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A PROCEDURE FOR CONCOMITANT PHOTOMETRIC ESTIMATION OF PREGNANEDIOL AND ESTROGENS IN HUMAN URINE*†

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BECAUSE of the intimate interrelationship of estrogen and progesterone metabolism to human reproductive physiology, there is need for a procedure which provides concomitant colorimetric pregnanediol and estrogen determinations in single twenty-four-hour urine specimens. Furthermore, since the sulfuric acid color reaction by which pregnanediol usually is determined is not specific for this steroid, incorporation of procedural modifications which contribute to more adequate characterization of the final pregnanediol sulfuric acid color product is highly desirable. This has been accomplished by the utilization of a liquid chromatogram technique which fractionates dihydroxysteroids from mono- and trihydroxysteroids, and by special attention to the spectral character of the pregnanediol sulfuric acid color reaction.

Received for publication August 30, 1951.

* A preliminary report of part of this study was read by title at the 29th Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, June 6, 1947, and abstracted in *J. Clin. Endocrinol.* **7**: 457, June, 1947.

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In our preparation of urinary phenol-like residues for chromatographic fractionation and photometric estimation of the estrogens (estrone, estradiol, and estriol) a neutral moiety is obtained (1). This crude neutral steroid residue may be utilized for colorimetric, *i.e.*, concentrated sulfuric acid, pregnanediol determination in a procedure in which a liquid chromatogram technique similar to that described by us (1, 2) for the natural estrogens has been incorporated. Umberger and Curtis (3) have shown that the absorption curves obtained by heating the natural estrogens with 90 per cent sulfuric acid for twelve minutes at 100° C. are sufficiently characteristic for each to serve as a means of identification. Our somewhat similar study of a number of neutral steroids which, like pregnanediol, produce color with concentrated sulfuric acid at room temperature has shown that the absorption maximum (420 mμ) for pregnanediol is sufficiently characteristic within the group of neutral steroids investigated, to aid in the identification of pregnanediol. By our procedure, as little as 1 mg. of pregnanediol added to 1 liter of men's urine immediately after hydrolysis, or from 2 to 10 mg. of pure sodium pregnanediol glucuronidate added immediately before hydrolysis, can be recovered as pregnanediol in satisfactory yields. A description of the procedure and of some preliminary tests of its potentialities and limitations forms the subject of this report. The application of this procedure to a study of simultaneous estrogen and pregnanediol excretion throughout the normal menstrual cycle will form the subject of a future report.

APPARATUS AND MATERIALS

All-glass apparatus and connections were used throughout the procedure.

Photometric equipment consisted of the Evelyn photoelectric colorimeter with an accompanying set of light filters (420 mμ and 520 mμ) and the Beckman DU quartz spectrophotometer with matched 1-cm. Corex cells.

The chromatographic apparatus consisted of a 13×300-mm. Pyrex glass tube with a fritted glass filter (coarse) sealed 4.0 cm. from the bottom end, which was attached to a modified vacuum adaptor by a 12/18 glass joint. A cylindrical funnel (48×100 mm.) was attached to the top of the tube by means of a 14/20 glass joint.

Flat-bottom, short-neck flasks (250 ml., 24/40 glass joint) were used to collect the filtrate.

Activated alumina, Alorco F-20 minus 80 mesh (Aluminum Ore Co., East St. Louis, Ill.) was further screened to 100–200 mesh as previously described (1).

Carbon, decolorizing (Norit-A) neutral (Eimer & Amend, New York) was used. A 250-ml. centrifuge bottle was filled one-quarter full with

Norit-A and then completely filled with benzene. After vigorous stirring, the bottle and contents were centrifuged for fifteen minutes at 1,500 r.p.m. and the supernatant liquid decanted. The Norit-A was then dried by storing for twenty-four hours at 38° C.

Ethyl ether U.S.P. was shaken with 1 per cent aqueous solution of ferrous sulfate, distilled, and stored in the refrigerator until used.

All organic solvents were redistilled in all-glass distillation units before use.

Sodium pregnanediol glucuronidate was freed from ketonic glucuronides by the method of Sutherland and Marrian (4). The ketone-free (Zimmermann test (5)) preparation used in these experiments melted at 281° C. (uncorrected).[1] Pregnane-3α,20(α)-diol was prepared from pure sodium pregnanediol glucuronidate by enzymatic (ox-spleen β-glucuronidase) hydrolysis and purified by chromatography. Our preparation of pregnane-3(α),20(α)-diol melted at 234° C. Other steroids were checked by melting point determinations and used as received.

EXPERIMENTAL

Photometric density of yellow color produced by steroids with concentrated sulfuric acid at room temperature. Since the pregnanediol-sulfuric acid color reaction proceeds at room temperature and becomes stabilized in twenty to thirty minutes, we tested a number of pure neutral steroids which might be present in crude steroid extracts of hydrolyzed urine, for color development under the same conditions. In the series of 16 steroids studied, pregnane-3(α),20(α)-diol and pregnane-3(β),20(β)-diol were fifth and sixth respectively in photometric density (420 mμ) by unit weight.[2] Compounds 11 to 16 inclusive (Table 1) showed less than 10 per cent of the photometric density (420 mμ) of pregnanediol and therefore have been ignored in subsequent treatment of the subject. These data serve to emphasize the observations of others (6–8) that the sulfuric acid color reaction for the determination of pregnanediol is highly nonspecific. The photometric density at 520 mμ has also been included in Table 1 to show that a high ratio of L-420/L-520 mμ cannot be relied upon for characterization of pregnanediol.

Behavior of pure neutral steroids in our liquid chromatogram technique. Since it was observed (1, 2) that among the natural estrogens (estrone, estradiol, and estriol) the trihydroxy estrogen (estriol) was most strongly, and the monohydroxy estrogen (estrone) was least strongly adsorbed on a column of activated alumina, a somewhat comparable adsorption rela-

[1] Melting points were determined in the Fisher Jones melting point apparatus.

[2] Evelyn photoelectric colorimeter.

tionship might be anticipated among the mono-, di-, and trihydroxy neutral steroids. Accordingly, 1-mg. to 30-mg. amounts of certain steroids (listed in Table 1) were individually tested in our liquid chromatogram technique. The filtrate fractions were collected in 20-ml. consecutive aliquots and tested for steroid content. As shown diagrammatically in Figure 1, all of the monohydroxy steroids tested, were eluted by 200 ml. of benzene. Pregnanediol was quantitatively eluted by 160 ml. of 2 per cent

TABLE 1. PHOTOMETRIC DENSITY* OF YELLOW COLOR DEVELOPED BY CRYSTALLINE STEROIDS (200γ) WITH CONCENTRATED SULFURIC ACID (10.0 ml.) AT ROOM TEMPERATURES FOR TWENTY MINUTES

Steroid No.	Compound	Photometric density	
		L-420 mμ	L-520 mμ
1	Δ^5-Androstene-3(β),17(α)-diol	0.846	0.332
2	Δ^5-Androstene-3(β),17(β)-diol	0.803	0.357
3	Dehydroisoandrosterone	0.570	0.074
4	Cholesterol	0.540	0.226
5	Pregnane-3(α),20(α)-diol	0.503	0.102
6	Pregnane-3(β),20(β)-diol	0.500	0.097
7	Δ^5-Androstene-3(β),16(β),17(α)-triol	0.444	0.086
8	Androstane-3(β),17(β)-diol	0.364	0.297
9	Androstane-3(β),16(α),17(α)-triol	0.300	0.108
10	Androstane-3(α),17(β)-diol	0.164	0.053
11	Pregnanol-3(α)-20-one (640γ)	0.055	0.013
12	Androsterone	0.050	0.026
13	Allo-pregnane-3(β),20(β)-diol	0.040	0.004
14	Allo-pregnane-3(β)-ol-20-one	0.018	0.000
15	Allo-pregnanedione	0.008	0.000
16	Pregnanedione	0.001	0.000

* Evelyn photoelectric colorimeter.

methanol-benzene. The C-19 diols (Table 1, Nos. 1, 2, 7, 9) also were eluted by 160 ml. of 2 per cent methanol-benzene, whereas the C-19 triols (Table 1, Nos. 6 and 8) remained on the column until eluted by 200 ml. of 10 per cent methanol-benzene. Thus, it appeared feasible by this relatively simple chromatographic technique to separate pregnanediol from all of the steroids studied in the sulfuric acid color reaction (Table 1) except the C-19 diols. It may be possible to develop more highly refined chromatographic procedures which will fractionate the C-19 from the C-21 diols but no simple quantitative technique appears to be available at present.

Spectral absorption characteristics of sulfuric acid color reaction with neutral steroids. Since it has been shown that the pregnanediol-sulfuric acid color reaction is highly nonspecific and that our liquid chromatogram technique has definite limitations in its capacity to fractionate pregnanediol from other steroids, *i.e.*, C-19 diols, a study was made of the spectral absorption characteristics (350 to 520 mμ) of the sulfuric acid color reaction of the neutral steroids, in the hope that pregnanediol might

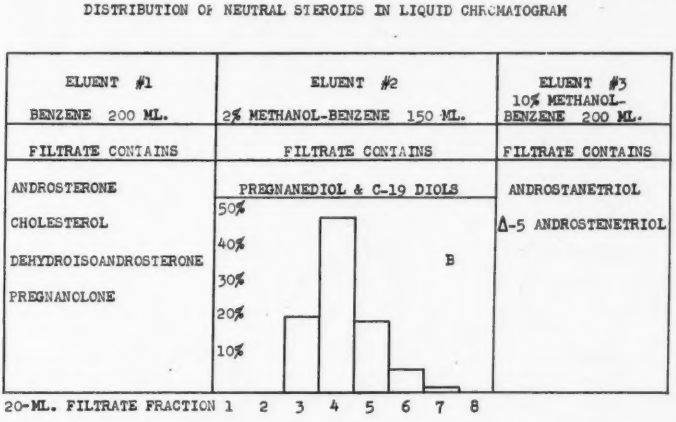

FIG. 1. Diagrammatic representation of elution of mono-, di-, and trihydroxy steroids in our liquid chromatogram technique. (B) Percentage distribution of 1.2 mg. of pregnane-3(α),20(α)-diol in 20-ml. aliquots of 2% methanol-benzene filtrate, following adsorption on chromatographic column and elution with benzene (200 ml.) and 2% methanol-benzene (160 ml.).

have some distinguishing characteristic which would aid in its identification. For convenience we have divided our series of absorption curves into 2 categories, 1) those compounds which can (Fig. 2) and 2) those compounds which cannot (Fig. 3) be separated from pregnanediol by means of our liquid chromatogram technique. It is noteworthy (Fig. 2) that pregnanol-3(α)-one-20 shows practically no absorption at wave lengths above 380 mμ and, as has been noted by Sommerville *et al.* (8), should not introduce any serious error in the pregnanediol determination unless present in abnormally large amounts such as might be found in human pregnancy urine. On the other hand, dehydroisoandrosterone is a powerful chromogen in the spectral range (400 to 450 mμ) commonly utilized for pregnanediol determinations. Also certain triols (Table 1, Nos. 6 and 8) which might be present in urine from patients with hyperadrenalism, are strongly chromogenic in the same spectral range. The same may be said

for the C-19 diols (Fig. 3). Inspection of all the spectral absorption curves, especially those in Figure 3, reveals that pregnanediol alone of all the neutral steroids studied by us exhibits a higher spectral absorption at 420 mμ than at 410 mμ.[3] We, therefore, have tentatively adopted as a criterion for urinary pregnanediol or pregnanediol-like residues, a spectral ratio of 420 mμ/410 mμ greater than 1. The validity of this criterion rests on the

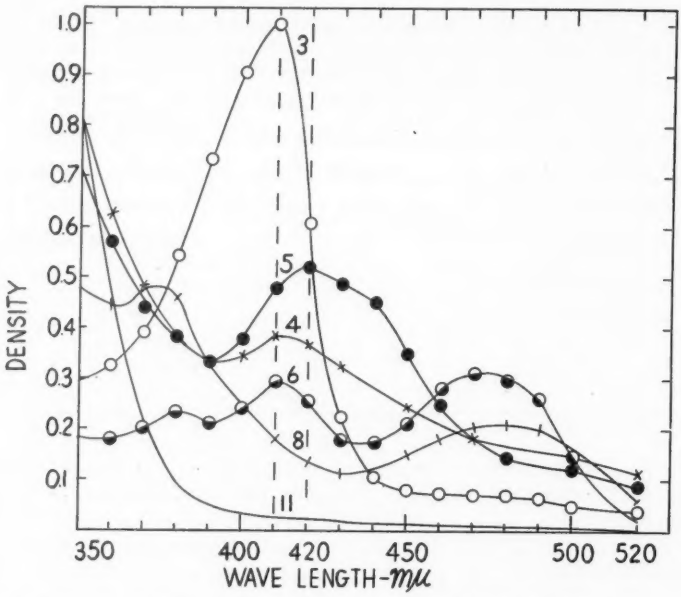

FIG. 2. Spectral absorption curves (200 micrograms) of sulfuric acid color tests of steroids which can be separated from pregnane-3(α),20(α)-diol by our liquid chromatogram technique. #5,pregnane-3(α),20(α)-diol (400-microgram curve included in this figure for reference); #3, dehydroisoandrosterone; #4, cholesterol; #6, Δ^5-androstene-3(β),16(β),17(α)-triol; #8, androstane-3(β),16(α),17(α)-triol; #11, pregnanol-3(α),-one,20-(640 micrograms).

assumption that twenty-four-hour aliquots of essentially pregnanediol-free men's urine should give by our procedure either no color at all in the sulfuric acid color reaction, or color with a spectral ratio of 420 mμ/410 mμ less than 1. A series of tests on essentially pregnanediol-free men's urine by the procedure to be described has supported this assumption.

[3] Since pregnane-3(α),20(α)-diol and pregnane-3(β),20(β)-diol exhibited similar spectral absorption curves in the sulfuric acid color reaction, the term pregnanediol may apply to either of the 2 isomers.

Method adopted for the determination of pregnanediol in urine. Numerous preliminary experiments, not recorded here, were conducted to test the minimal amount of procedural detail necessary in conjunction with the liquid chromatogram technique, in order to give consistently satisfactory results with tests for pregnanediol. The procedure finally adopted in-

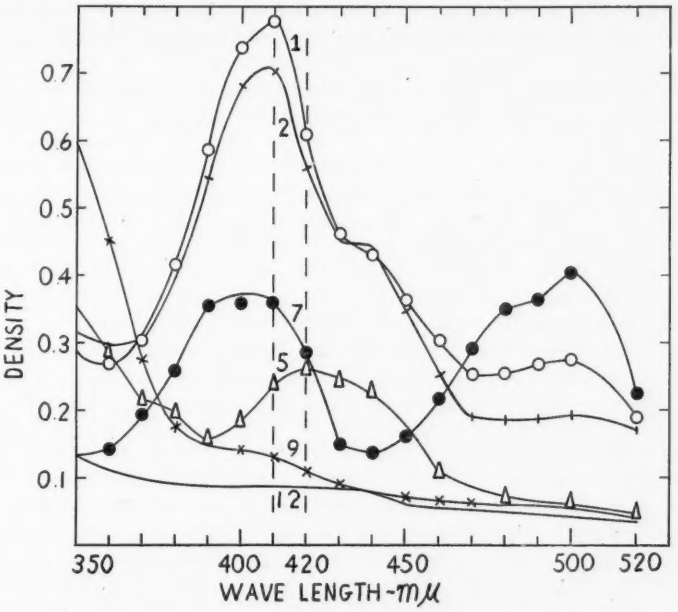

FIG. 3. Spectral absorption curves (200 micrograms) of sulfuric acid color tests of steroids which cannot be separated from pregnane-3(α),20(α)-diol by our liquid chromatogram technique. #1, Δ^5-androstene-3(β),17(α)-diol; #2, Δ^5-androstene-3(β),17(β)-diol; #7, androstane-3(β),17(β)-diol; #5, pregnane-3(α),30(α)-diol; #9, androstane-3(α),17(β)-diol; #12, pregnanediol-like residue from 1 liter of men's urine.

corporates many of the suggestions of Sommerville *et al.* (8) relative to the "Astwood-Talbot" method.

A twenty-four-hour specimen of urine collected with 100 ml. of toluene as a preservative is acidified with 15 vols. per cent of concentrated hydrochloric acid and boiled under reflux for twenty minutes. The flask is then cooled rapidly under running tap water and the contents transferred to an extraction chamber which has been previously described (1). The supernatant toluene (100 ml.) is drawn off and the urine is returned to the extraction chamber and extracted four times by mechanical stirring with one-quarter volume of ethyl ether-toluene mixture (9:1). The combined clear organic extracts (toluene and ether-toluene) are decanted into a three-liter flask and later enriched with the organic phase obtained from centrifugation of the emulsion which usually accompanies the

extraction process. The solvent mixture is then concentrated by evaporation on a hot water bath until ether distillation ceases. The residual toluene extract (200 to 300 ml.) is immediately cooled under running tap water and transferred to a 500-ml. separatory funnel. It is then extracted three times with 100 ml. and once with 50 ml. of 1 N aqueous sodium hydroxide, which previously has been used to wash out the evaporating flask. The combined alkaline aqueous extracts are made acid to litmus paper with concentrated hydrochloric acid and utilized for the preparation of phenol-like residues as previously described (1). The chromatographic fractionation and photometric estimation of the estrogens are carried out on this phenol-like residue by previously described procedures (1, 2).

Further purification of the alkali-washed toluene extract. Approximately 2.0 grams (half of a teaspoonful) of sodium hydrosulfite ($Na_2S_2O_4$) powder and 50 ml. of aqueous 1 N sodium hydroxide are added to the toluene extract and the mixture shaken vigorously until the purple or red color changes to yellow. The aqueous phase is discarded together with two 50-ml. washings of the toluene extract with distilled water. (The extract should be neutral to litmus at this point.) The toluene extract is then evaporated to dryness under reduced pressure on a hot water bath. The residue is redissolved in 0.6 ml. of absolute methanol, diluted to 30 ml. with benzene, and reserved for the liquid chromatogram. (A convenient aliquot, 0.01 of the total, can be evaporated and reserved for determination of neutral 17-ketosteroids by the Zimmermann test (5).)

Preparation of liquid chromatogram. The chromatographic apparatus is assembled and attached to the house vacuum line which is in the "off" position. Sixty ml. of benzene are added to the column and 17 Gm. of alumina are quickly dumped into the benzene reservoir. The vacuum line is turned on and the side of the column tapped vigorously while the alumina settles to the bottom of the column. The column should settle to a height of 22 to 23 cm. The solution containing the neutral steroid residue is added to the column as soon as the benzene has drained from the cylindrical funnel. A 20-ml. aliquot of the succeeding 200 ml. of benzene eluent is used to wash out the flask and is poured into the column immediately following drainage of the neutral steroid solution from the funnel. The remaining benzene (180 ml.) is then added to the column. The filtrates from the steroid solution and the 200-ml benzene eluent are discarded. The 2 per cent methanol-benzene eluent (150 ml.) is then poured into the column and the filtrate therefrom is evaporated under reduced pressure on the hot water bath to a volume of 30–40 ml.[4] Approximately 300 mg. of Norit-A are added to the benzene solution while the solution is still hot. After the Norit settles, the supernatant benzene solution is decanted into a Buchner-type filter funnel with a fritted glass disc (60 ml., medium porosity) and filtered under reduced pressure. The Norit residue is washed twice with 5.0 ml. of benzene and the combined filtrates evaporated to dryness under reduced pressure. Five ml. of 95 per cent ethyl alcohol are added to the flask and evaporated similarly to remove final traces of benzene. The residue is redissolved in 10 ml. of 95 per cent ethyl alcohol and diluted with 40 ml. of distilled water at 90–100° C. The flask is stoppered and stored at room temperature over night. Without further cooling, the pregnanediol precipitate is collected on a Buchner-type fritted glass filter funnel (60 ml., medium porosity) and the precipitation flask and funnel washed twice with 10 ml. of distilled water. The precipitate is then dissolved in 20 ml. of hot (70–78° C.) 95 per cent alcohol and the solution collected in the same flask in which the pregnanediol was precipitated. After being diluted

[4] Elution of the chromatographic column beyond this point is not practiced in pregnanediol determination.

to definite volume, suitable aliquots of the pregnanediol solution are evaporated to dryness in the hot water bath in colorimeter tubes, preparatory to addition of the concentrated sulfuric acid color reagent.

Development and measurement of sulfuric acid color reaction. For color development the method of Talbot *et al.* (6) has been used without modification, *i.e.*, 10 ml. of concentrated sulfuric acid are added to the colorimeter tube containing the pregnanediol residue and the mixture stirred intermittently with a glass stirring rod for twenty minutes. The color density is measured in the Evelyn photometric colorimeter, using a 420 mμ filter. The amount (mg.) of pregnanediol in the sample is calculated from the equation of Talbot *et al.* (6). An aliquot of the sulfuric acid color reaction mixture is then transferred to a Corex cuvette and its color absorption spectrum scanned at 10-mμ intervals between 400 and 450 mμ in the Beckman DU spectrophotometer.

Recovery experiments. A series of tests on 1-liter aliquots of different batches of pooled men's urine by our procedure consistently yielded putative pregnanediol titers ranging from 0.027 to 0.300 mg. per liter. All of these tests have yielded yellow colors which showed spectral absorption characteristics (ratios of 420 mμ/410 mμ less than 1) similar to curve 12 in Figure 3. The addition of 1-mg. to 10-mg. amounts of pregnanediol to 1 liter of pooled men's urine immediately following acid hydrolysis, yielded 90 to 110 per cent recovery of pregnanediol (Table 2, experiments 6 and 7). As little as 0.4 mg. of added pregnanediol gave a pregnanediol-type of spectral ratio (420 mμ/410 mμ greater than 1) but was not quantitatively recovered (Table 2, experiments 4 and 5), whereas 0.2 mg. of pregnanediol or less yielded neither satisfactory spectral ratio nor recovery.

Recovery experiments with known amounts of pure sodium pregnanediol glucuronidate (Table 2) added to 1-liter aliquots of pooled men's urine immediately before acid hydrolysis, have not in our experience yielded pregnanediol recoveries exceeding 80 per cent of the theoretical (56 per cent) pregnanediol content. However, the addition of 5-mg to 10-mg. amounts of sodium pregnanediol glucuronidate per liter of men's urine consistently yielded 70 to 80 per cent recoveries under the most favorable conditions of acid hydrolysis, *i.e.*, 10 to 15 volumes per cent of concentrated hydrochloric acid and ten to fifteen minutes' boiling under reflux. The major portion of the 20 to 30 per cent loss of pregnanediol from sodium pregnanediol glucuronidate is not due to contamination of our sodium pregnanediol glucuronidate with pregnanolone glucuronidate but probably is due to destruction during the hydrolytic procedure.[5] We have included in Table 3 the data from a number of recovery tests of putative pregnanediol from men's urine to which steroids which might give false-positive findings with the pregnanediol tests, were added. The results of

[5] Studies are in progress with enzymatic hydrolysis in an attempt to throw some light on the loss of pregnanediol glucuronidate incurred by our procedure.

TABLE 2. RECOVERY OF PREGNANEDIOL AFTER THE ADDITION TO 1-LITER ALIQUOT OF POOLED MEN'S URINE OF (A) PREGNANEDIOL, (B) SODIUM PRENANEDIOL GLUCURONIDATE, (C) SUBSTANCES WHICH MIGHT INTERFERE WITH COLORIMETRIC DETERMINATIONS

Experiment No.	Amount added, mg.		Pregnanediol recovery (corrected for endogenous titer*)		Spectral absorption ratio, 420 mμ / 410 mμ
			mg.	%	
		A			
1	0.10	Added after hydrolysis	0.044	44	<1.0
2	0.20	Added after hydrolysis	0.028	14	<1.0
3	0.20	Added after hydrolysis	0.059	29	<1.0
4	0.40	Added after hydrolysis	0.252	46	>1.0
5	0.60	Added after hydrolysis	0.470	66	>1.0
6	1.08	Added after hydrolysis	1.150	106	>1.0
7	3.16	Added after hydrolysis	3.09	98	>1.0
		B			
8	2.0	Added before hydrolysis	0.645	58	>1.0
9	2.0	Added before hydrolysis	0.805	72	>1.0
10	6.0	Added before hydrolysis	2.41	72	>1.0
11	10.0	Added before hydrolysis	3.96	71	>1.0
12	10.0	Added before hydrolysis	4.26	79	>1.0
		C			
13	20.0	Dehydroisoandrosterone acetate†	0.044	—	<1.0
14	50.0	*dl*-α-Tocopherol acetate (synthetic)†	0.000	—	—
15	50.0	Cortisone acetate (synthetic)†	0.016	—	<1.0

* Average endogenous titer for 1 liter of men's urine = 0.060 mg.
† Added before hydrolysis.

these tests were essentially negative. It is especially noteworthy that as much as 20 mg. of the highly chromogenic dehydroisoandrosterone added to one liter of men's urine does not abnormally elevate the putative pregnanediol titer by our procedure.

Preliminary clinical studies. In order to test the capacity of the proposed pregnanediol procedure to elicit significant information regarding the metabolism of therapeutic doses of progesterone and other potential pregnanediol precursors, a number of preliminary experiments were carried out. It can be seen (Table 3) that a single injection of the usual therapeutic dose (25 mg.) of progesterone yields measurable amounts of urinary preg-

nanediol in the forty-eight-hour postinjection period, by our procedure. The full potentialities of the procedure are better illustrated in Experiment 2, in which concomitant exogenous pregnanediol and estrogens[6] were measured following therapeutic doses of progesterone and estrogen (α-estradiol).

Since massive doses of desoxycorticosterone acetate had yielded measurable amounts of urinary pregnanediol (1 to 3 per cent) in man (10), we have included in Table 3 the negative results of our preliminary test for

TABLE 3. PRELIMINARY EXOGENOUS URINARY PREGNANEDIOL AND ESTROGEN EXCRETION STUDIES IN MEN (48-HOUR POST-TREATMENT URINE)

Experiment No.		Pregnanediol			Estrogen*		
		mg.	%	420 mμ / 410 mμ	O	D	T
1	25 mg. Progesterone (oil, I.M.)	4.25	17.0	>1.0	6	0	0
2	25 mg. Progesterone (oil, I.M.) and 3.0 mg. α-estradiol (oral)	1.93	7.7	>1.0	172	16	16
3	20 mg. Desoxycorticosterone acetate (oil, I.M.)	0.08		<1.0	not determined		
4	300 mg. *dl*-α-Tocopherol acetate (synthetic, oral)	0.20		<1.0	7	0	8
5	100 mg. Cortisone acetate (saline suspension I.M.)	0.71		<1.0	6	0	4
6	60 mg. Adrenocorticotropic hormone (I.M., 10 mg. 6× daily) (Armour)	0.226		<1.0	4	0	0

* O—Estrone. D—Estradiol. T—Estriol.

pregnanediol excretion following treatment with a single 20-mg. dose of desoxycorticosterone. Additional tests will be necessary to establish the threshold for measurable pregnanediol excretion by this procedure.

There is conflicting evidence that vitamin E (*dl*-α-tocopherol) exerts its antisterility effect by its action on progesterone metabolism (11). Our single experiment with 300 mg. (orally, in a dose of 50 mg. six times daily) of synthetic *dl*-α-tocopherol yielded negative findings with the pregnanediol test in the forty-eight-hour post-treatment urine.

Because pregnanediol has been isolated from the urine of persons with adrenal hyperfunction (12) it seems of interest to report our failure (single study) to find measurable amounts of pregnanediol by our procedure in

[6] A color correction equation (9) was used for calculation of estrogen titer.

the forty-eight-hour urine collected immediately following injection of 60 mg. (10 mg., six times daily) of adrenocorticotropic hormone (Table 3, experiment 6).

DISCUSSION

Existing methods for urinary pregnanediol extraction commonly employ solvents (toluene, carbon tetrachloride, etc.) which possess unfavorable solvent/water partition ratios with respect to the natural estrogens, especially to the strongly hydrophilic estriol. Consequently they are unsuitable for concomitant estrogen and pregnanediol extraction. On the other hand, ethyl ether which is an equally good solvent for the extraction of pregnanediol as well as the natural estrogens yields urinary neutral steroid residues which, in the case of pregnanediol, are quite highly contaminated by interfering pigments and chromogens. Therefore, in order to retain the quantitative extraction features of our procedure for the urinary estrogens we have employed the 9:1 ethyl ether-toluene mixture, which later in the procedure is evaporated until essentially free from ether. Since toluene also provides a more satisfactory organic phase than ether for quantitative extraction of the estrogens by aqueous sodium hydroxide (13), this modification serves a dual purpose of providing cleaner neutral steroid residues and more quantitative recovery of estrogens.

We have adopted the Astwood and Jones (14) suggestion for simultaneous hydrolysis and toluene extraction with the 100 ml. of toluene used as a preservative, although our data (unpublished) do not support the concept that pregnanediol recoveries thereby are enhanced (14).

In order to free our neutral steroid residue from purple or red pigments commonly encountered in hydrolyzed urine extracts, we have utilized a modification of the sodium hydrosulfite treatment of Talbot *et al.* (15). The residual yellow pigments are weakly adsorbed on the chromatographic column and consequently are eluted by the 200 ml. of benzene. Even so, a more strongly adsorbed faint orange colored pigment is observed in our 2 per cent methanol-benzene filtrate fraction after concentrating almost to dryness. We have adopted the Norit-A treatment of Sommerville *et al.* (8) to remove this last trace of pigment.

Sommerville *et al.* (8) have investigated extensively the effect of cooling upon the amount and character of pregnanediol precipitated in 20 per cent aqueous alcohol solution. We have found that the final cooling in the refrigerator is of dubious value, since it frequently results in turbid solutions which are difficult to filter and occasionally leads to adherence of the precipitate to the sides of the flask. We therefore have adopted as routine procedure, storing the precipitation flask over night at room temperature. We also find no necessity for controlling the temperature of color develop-

ment with sulfuric acid under the climatic conditions which prevail in this area.

Our criterion for colorimetric characterization of pregnanediol, *i.e.*, a sulfuric acid color absorption ratio of 420 mμ/410 mμ greater than 1, is a rather sharp one, since pure pregnane-3(α),20(α) and pregnane-3(β), 20(β)-diol give a ratio of only 1.1. Therefore, the Beckman DU quartz spectrophotometer or one with comparable definition of wave length should be used for scanning. Our absorption curve for pregnanediol is similar to that published by Guterman and Schroeder (7). However, for their spectrophotometric data, instead of men's urine they used pooled women's urine which may have contained pregnanediol. This may account for the pregnanediol-like curve which they reported for untreated urine.

It is recognized by us that the 16 neutral steroids studied comprise only a part of the total number potentially available from acid-hydrolyzed human urine. We, therefore, have considered our criterion for a satisfactory pregnanediol-test result (spectral ratio 420 mμ/410 mμ greater than 1) as a tentative one. Other neutral steroids which produce a yellow color at room temperature with concentrated sulfuric acid will be studied in this respect as they become available to us.

SUMMARY

Our procedure for the fractionation and photometric estimation of urinary estrogens has been modified so as to give concomitant colorimetric pregnanediol estimation. The specificity of the sulfuric acid color reaction for pregnanediol has been improved by specifying a spectral absorption ratio of 420 mμ/410 mμ greater than 1, for positive identification. Pregnanediol in amounts ranging from 1 to 10 mg. per twenty-four-hour urine specimen can be satisfactorily determined. Some preliminary clinical studies with single therapeutic doses (25 mg.) of progesterone have demonstrated the utility of the procedure.

Acknowledgments

We wish to thank the following individuals and firms for their courtesy in supplying certain materials to us for this study: Dr. Gordon A. Grant, of Ayerst, McKenna, and Harrison Ltd., Montreal, Canada, for the sodium pregnanediol glucuronidate; Dr. Thomas F. Gallagher, Sloan-Kettering Institute for Cancer Research, New York City, for pregnanol-3(α)-one-20; Dr. Max N. Huffman, Oklahoma Medical Research Institute and Hospital, Oklahoma City, Oklahoma, for androstane-3(β),16(α),17(α)-triol and Δ^5-androstene-3(β),16(β),17(α)-triol; and Dr. Ernest Oppenheimer of Ciba Pharmaceutical Products, Inc., Summit, N.J., for androstane-3(β),17(β)-diol, androstane-3(α),17(α)-diol, Δ^5-androstene-3(β),17(β)-diol, Δ^5-androstene-3(β),17(α)-diol, pregnane-3(β),20(β)-diol, allo-pregnane-3(β),20(β)-diol, pregnanedione, allo-pregnanedione, and allo-pregnane-3(β)ol-20-one.

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THE QUANTITATIVE ASSAY OF SERUM CHORIONIC GONADOTROPIN IN PREGNANCY, USING THE MODIFIED MALE FROG TECHNIQUE

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THE discovery of chorionic gonadotropin and the elaboration of various laboratory procedures for its detection and mensuration led to many published observations concerning the occurrence of the hormone as well as the establishment of quantitative norms in pregnancy and the amounts present in certain pathologic conditions occurring in humans. In most instances these values lack universal applicability because of the diversity of unitage assigned to the hormone in the several institutions in which the observations were made. The direct transference, for example, of a mouse-uterus unit to a rabbit unit or an ovarian-hyperemia unit is not feasible from a practical standpoint.

With the introduction of the male frog test for pregnancy at this institution and the subsequent modification of the test for quantitative procedures, it was considered expedient to adopt the practice of reporting results as International Units of chorionic gonadotropin per cubic centimeter of blood serum. This precluded the need for establishing still another unit of chorionic gonadotropin. It further provided an adequate means for comparison of our findings with those of other experimenters using International Unitage for mensuration.

The adoption of the frog technique for the quantitative assay of chorionic gonadotropin necessitated the re-evaluation of norms previously established by other methods or the establishment of a new series of norms prior to the assignation of significance to any single value obtained by the new method. When this study was instituted, data on serum chorionic gonadotropin levels, expressed in International Units, were not readily available. It was therefore considered advisable to embark upon a study to establish serum chorionic gonadotropin values during pregnancy, expressed in International Units, for this particular bio-assay technique.

The purpose of this report is to present titers of serum chorionic gonadotropin in 57 patients during the course of pregnancy. In addition some observations made on patients with abnormalities of gestation are presented.

METHOD

All chorionic gonadotropin studies were carried out, using blood serum

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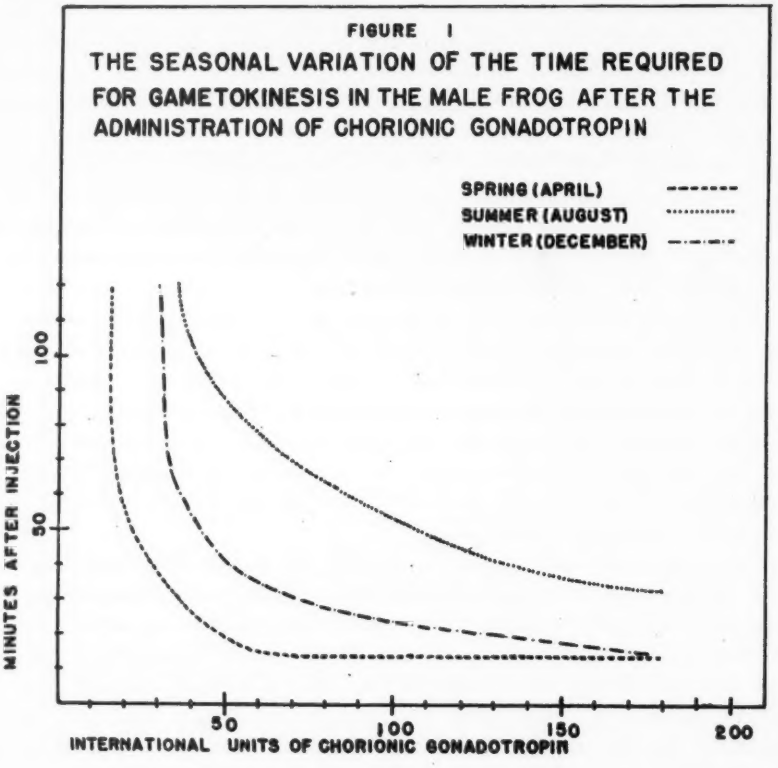
as the substance tested. The modification of the male frog test for pregnancy used for the assay of chorionic gonadotropin was that described earlier (1). The method is based on the relationship between the amount of chorionic gonadotropin administered to the male frog (*Rana pipiens*) by intraperitoneal injection, and the time required for gametokinesis to occur.

As the study progressed it became apparent that the gametokinetic response of the frog to chorionic gonadotropin was subject to seasonal

FIGURE 1

THE SEASONAL VARIATION OF THE TIME REQUIRED FOR GAMETOKINESIS IN THE MALE FROG AFTER THE ADMINISTRATION OF CHORIONIC GONADOTROPIN



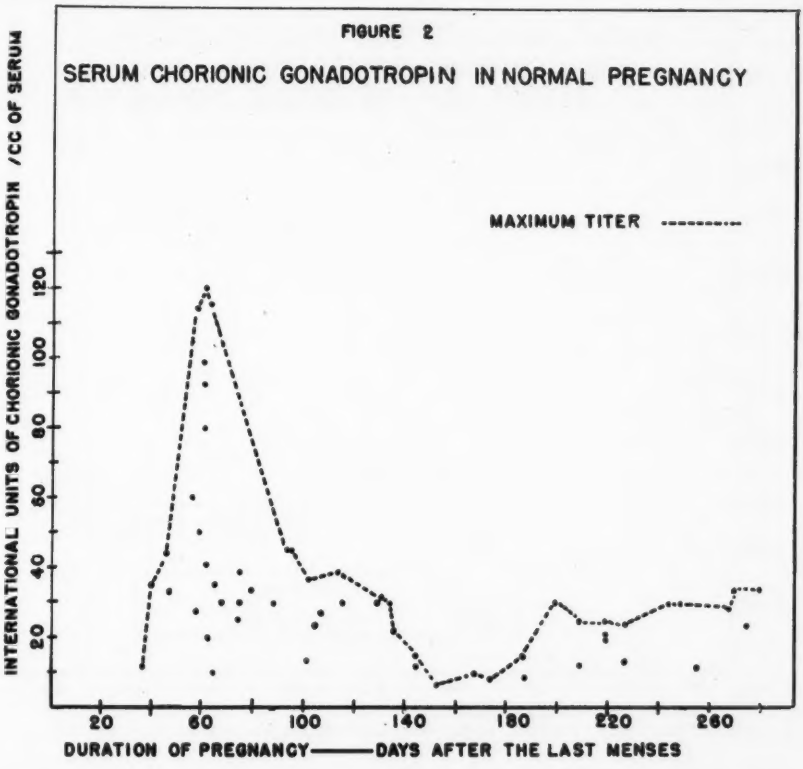
modification. The sensitivity of the frog (*Rana pipiens*) to chorionic gonadotropin was greatest in the spring and least in the late summer. This seasonal variation is depicted in Figure 1. It was obvious from this observation that accuracy of titration could only be assured by frequent standardization of animals used for testing.

In titrating a given serum sample, after locating the amount of serum necessary to place the time for gametokinesis on the optimum part of the curve, 4 animals were used for the final result. If dilution was necessary, distilled water was used as the diluent. The average time necessary for

gametokinesis to occur in 4 frogs was noted and applied to the standard curve currently in use. The dilution factor was then considered and the results reported in International Units of chorionic gonadotropin per cubic centimeter of serum.

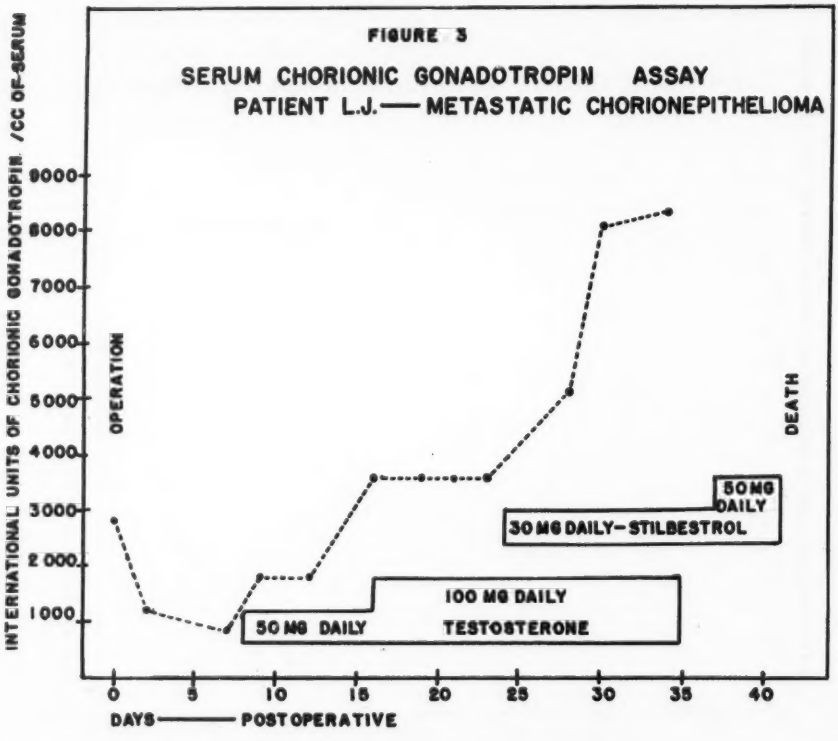
RESULTS

Serum chorionic gonadotropin titers were determined in 57 women whose period of gestation varied from 34 to 280 days after the last menstrual



period. Clinical appraisal of this group of women determined their pregnancies to be uncomplicated. The results of these observations are shown graphically in Figure 2, in which the maximum titers are plotted. The highest value was obtained at 62 days after the last menstrual period and was 120 I.U. of chorionic gonadotropin per cubic centimeter of serum. Following this there was a rapid decline in titer to the lowest recorded level at 154 days after the last menstrual period. Then a slight rise in titer was noted, until the termination of pregnancy.

The general characteristics of this chorionic gonadotropin curve in pregnancy do not vary significantly from those previously published. Wilson, Albert and Randall (2), using ovarian hyperemia in the rat as the endpoint in chorionic gonadotropin titration, have reported findings almost identical with those in Figure 2. Since these workers reported their results in International Units, direct comparison of the two series is possible and shows similarity of absolute values as well as of the characteristics of the

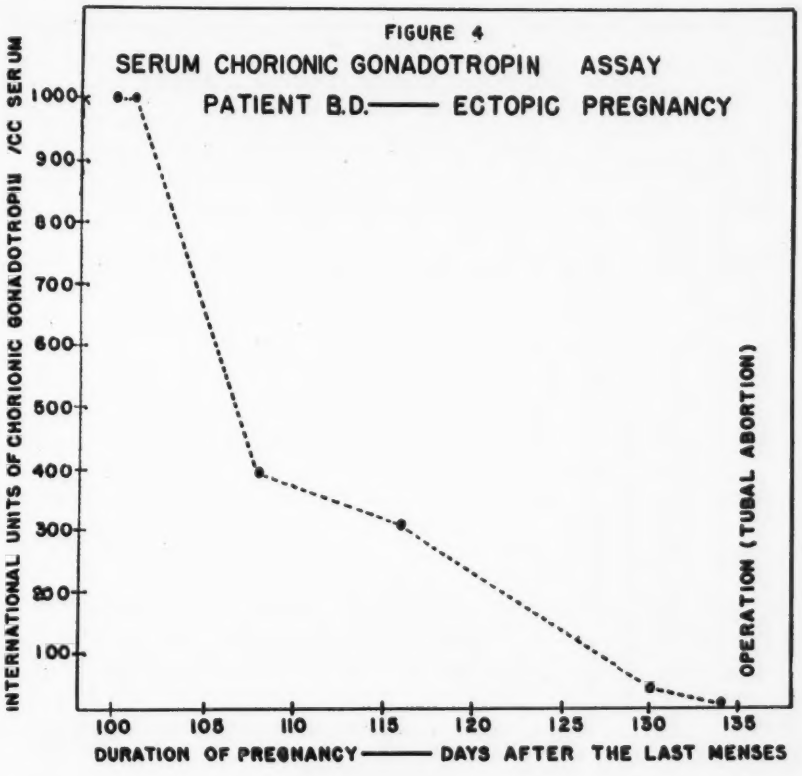


serum chorionic gonadotropin curve during pregnancy, despite the fact that different laboratory animals and different end organs were utilized in determining the presence of the hormone.

Several interesting patients with abnormalities of gestation, whose serum chorionic gonadotropin concentrations were considered worthy of note, were encountered during this investigation. Patient L. J. was operated upon with the diagnosis of ruptured tubal pregnancy. Laparotomy confirmed this impression but revealed, grossly and microscopically, chorionepithelioma at the site of rupture. Serum chorionic gonadotropin

assay was instituted on the day of surgery and was continued at frequent intervals, until the patient's demise on the forty-second postoperative day. Figure 3 indicates the results of these bio-assays.

Since it has been shown that estrogens and androgens are useful in the palliative therapy of certain metastatic malignancies and the administration of these hormones results in the depression of circulating pituitary

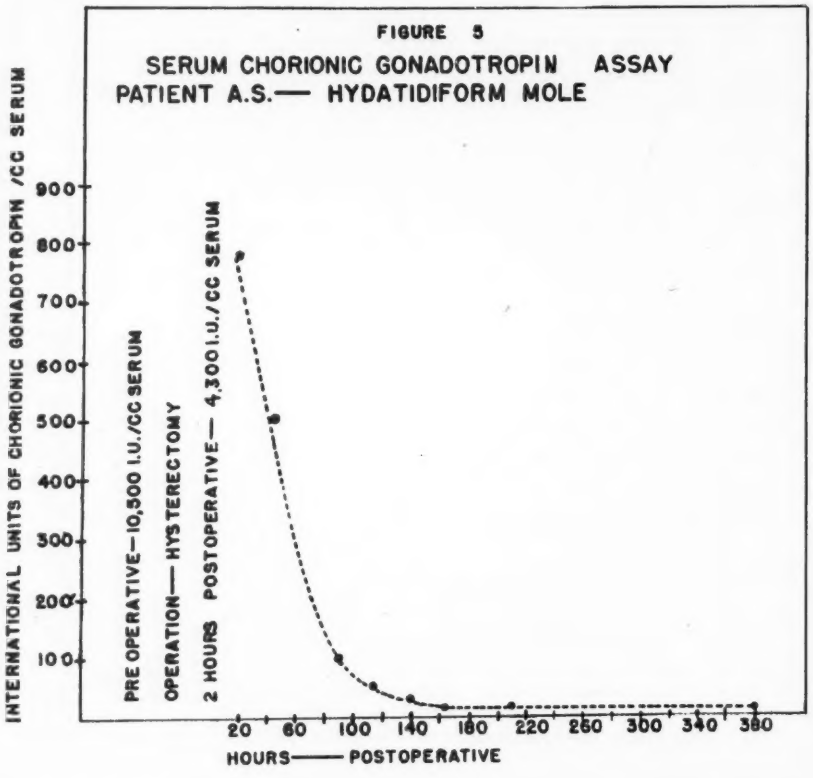


gonadotropin levels, it was decided to give stilbestrol and testosterone to this patient. The clinical course of this patient's illness was not demonstrably altered by the use of these two substances, either singly or in combination. As noted in Figure 3, the serum chorionic gonadotropin concentration continued to increase, despite the therapy, until the death of the patient.

Patient B. D. presented unusual findings from both the laboratory and clinical aspects. At an estimated 90 days of gestation she was operated

upon, with the provisional diagnosis of tubal pregnancy. At operation, bilateral adnexal masses associated with chronic inflammatory change and an enlarged uterus were thought to represent an intra-uterine pregnancy in the presence of chronic pelvic inflammatory disease. Accordingly, no pelvic surgery was carried out.

At an estimated 100 days of pregnancy, serum chorionic gonadotropin studies were instituted as indicated in Figure 4. An initial titer of 1,000



I.U. of chorionic gonadotropin was found and confirmed on the following day. The serum titer gradually fell to zero at 134 days of pregnancy. At this time further exploration by laparotomy revealed the presence of a dead fetus and placenta in the cul-de-sac. There was a right oviductal rupture. Histologic study of the operative specimen, including both tubes and the right ovary, failed to reveal any evidence of a chorionepithelioma or a hydatidiform mole. Postoperative study of the serum failed to reveal chorionic gonadotropin.

Patient A. S., a 29-year-old gravida-10, para-9, was admitted to the hospital with the diagnosis of hydatidiform mole. The initial titer of serum chorionic gonadotropin was 10,500 I.U. per cubic centimeter. The treatment by complete abdominal hysterectomy because of multiparity was accomplished without incident and afforded the opportunity of observing the disappearance of serum chorionic gonadotropin in the absence of the possibility of retained functioning intra-uterine chorionic tissue. The pattern of decline of the serum chorionic gonadotropin is illustrated in Figure 5. There was demonstrable hormone on the sixteenth postoperative day, with a titer of 19 I.U. per cubic centimeter of serum. A test made on the twenty-sixth postoperative day failed to reveal the presence of gonadotropic activity.

Several observations made in other patients with complications of gestation are listed in Table 1. The observed serum chorionic gonadotropin titers are compared with the titers from patients with normal pregnancies

TABLE 1. TITERS OF SERUM CHORIONIC GONADOTROPIN IN ABNORMAL PREGNANCIES

Patient	Diagnosis	Duration of pregnancy (days)	Titers of gonadotropin (I.U./cc.)	Percentage increase over normal
D. P.	Eclampsia	182	71	355
C. A.	Enzygotic twins	123	225	642
B. B.	Hydatidiform mole	81	224	300
E. A.	Chorionepithelioma	140	292	1,622
I. G.	Hydatidiform mole	102	2,600	6,500

at a similar period of gestation. The increase over normal was then tabulated as percentage increase in titer. Although no real significance is attached to these limited observations, it is apparent that in each case the increase in the titer was such that serum chorionic gonadotropin was considered present in abnormal amounts.

SUMMARY

The blood serum of 57 women with uncomplicated pregnancies has been quantitatively bio-assayed for chorionic gonadotropin, using a modification of the male frog test for pregnancy, and the findings reported in International Units. Several abnormalities of gestation in which serial serum chorionic gonadotropin studies were made are presented. These include ectopic pregnancy, chorionepithelioma and hydatidiform mole. Single observations on patients with twins, eclampsia, moles and chorionepithelioma are also reported. One patient with chorionepithelioma has

been treated with testosterone and stilbestrol without apparent beneficial effect on the clinical course of the disease or on the titer of serum chorionic gonadotropin. The seasonal variation of the male frog (*Rana pipiens*) in responding to chorionic gonadotropin is also discussed.

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MEASURMENTS OF ADRENAL CORTICAL ACTIVITY IN YOUNG MEN SUBJECTED TO ACUTE STRESS*†

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INTRODUCTION

ALTHOUGH there have been many studies of the response of animals and of human subjects to acute physiologic or psychologic stress, usually only a few of the indices of adrenal cortical function have been determined in any one experiment. In this study, we have attempted to identify the degree to which the adrenal cortex is affected in human subjects after exposure to physiologic stress of short duration, by a series of determinations before and for several hours after the stress. The procedures were selected on the basis of experimental reports which have indicated that these determinations reflect adrenal cortical function.

EXPERIMENTAL PROCEDURES AND METHODS

Twenty-one normal male subjects were immersed for a period of exactly eight minutes in water which had been cooled with ice to a temperature of 9.5° C. The subjects were completely immersed to neck level and allowed only minimal activity in the water. The water had been thoroughly mixed with electric mixers and was maintained at the stated temperature during the period of immersion.

Samples of venous blood were drawn immediately before, immediately after, and one, two and four hours following the immersion. A "timed" overnight urine sample was collected from the time of retiring until arising in the morning. A pre-immersion sample was taken from the time of rising until immediately before the immersion. Other urine samples were taken at the same time as the blood samples. At the time of each blood sample, the oral temperature, heart rate and blood pressure were noted.

The following determinations were made on the blood samples: counts of the total white cells, neutrophils and lymphocytes (by the usual method); the eosinophil count (by the method of Randolph (1)); the concentration of serum sodium and plasma potassium (on a flame photometer with an

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TABLE 1. MEANS AND STANDARD DEVIATIONS OF VALUES FOR BLOOD CONSTITUENTS BEFORE AND AFTER STRESS

	Number of subjects	Pre-stress		Immediately after stress		1 Hour after stress		2 Hours after stress		4 Hours after stress	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Body temperature (° F.)	21	98.6	1	97.5	1.4	97.6	.8	98	1.1	98.2	.5
Heart rate (per min.)	21	84	15	87	18	72	11	72	12	73	14
Systolic blood pressure (mm. Hg)	21	126	11	141	15	123	12	116	9	120	10
Diastolic blood pressure (mm. Hg)	21	77	11	86	11	77	8	71	9	73	8
Blood:											
White cell count (per cu. mm.)	21	7,500	1,950	8,200	2,250	8,300	2,300	9,000	3,700	8,400	1,850
Neutrophils (per cu. mm.)	21	4,100	1,600	4,500	1,550	5,100	2,150	6,100	3,400	5,100	1,700
Lymphocytes (per cu. mm.)	21	3,000	700	3,300	1,250	2,900	900	2,500	850	3,000	700
Eosinophils (per cu. mm.)	18	140	78	137	71	111	62	82	46	90	48
Sugar (mg. %)	18	86	8	87.5	8	82.6	6.3	84.2	6.1	82.5	5.6
Serum H_2O (Gm./Kg.)	18	933.3	3.7	929.7	3.6	931.8	3.3	931.4	3.6	932.1	4.2
Sodium (mEq./L.)	21	140.6	3	141.4	3.4	140.5	3.6	140.4	3.5	140	4
Potassium (mEq./L.)	20	3.89	.33	3.99	.38	4.10	.40	4.04	.42	3.95	.30
Chloride (mEq./L.)	19	105.4	2.0	104.1	2.1	104.6	2.1	104.3	1.7	105.0	2.1
Total protein (Gm. %)	18	7.36	.53	7.79	.58	7.57	.63	7.55	.65	7.54	.54
Ascorbic acid (mg. %)	21	.89	.39	.92	.42	.88	.40	.89	.42	.91	.41
Total cholesterol (mg. %)	19	192	47	211	49	197	54	201	51	204	52
Free cholesterol (mg. %)	19	48	14	53	18	49	17	50	18	50	17
Cholesterol esters (mg. %)	19	149	30	161	33	148	37	152	35	155	36

internal standard); serum chloride (by a modification of Sendroy's method (2)); total serum protein (method of Howe (3)); serum water (gravimetrically (4)); whole blood sugar (Nelson's modification of Somogyi's method (5)); ascorbic acid (method of Roe and Kuether (6)); serum cholesterol and free cholesterol (modification of the method of Schoenheimer and Sperry (7)). In the urine samples, the excretions of sodium, potassium, chloride, ascorbic acid, uric acid (Benedict and Frank (8)) and creatinine (Folin (9)) were determined.

The excretion of 17-ketosteroids was determined on the overnight urine specimen collected on arising in the morning (sleep specimen), on the urine voided from this time until just before the stress, and on the combined urine samples collected for the first two hours and the succeeding two hours after the stress. The determinations of 17-ketosteroids were performed on aliquots of each collection corresponding to approximately one hour's excretion of urine, except for the overnight specimen from which a $3\frac{1}{2}$-hour aliquot was used. The urine was hydrolyzed by refluxing for ten minutes in 5 per cent sulphuric acid; it was then extracted with ether, and the ether washed with 2 N sodium hydroxide and water. Assays on the resulting neutral fraction were performed by the Callow procedure (10), using stabilized alcoholic potassium hydroxide (11). The results are calculated as milligrams of dehydroisoandrosterone equivalents per hour.

The means and standard deviations were computed for each of the blood and urine constituents determined in the 21 subjects before, immediately after, and one, two and four hours after stress. To test the effects of stress, the values of the data before and after stress were used. For each subject, the pre-stress level of each determination was subtracted from its level after stress. The averages of the differences were plotted and the *t*-test for significance of paired differences was applied. When this test showed a difference which would occur less than 5 times in 100 samples, that point is starred on the graphs, in order to indicate a significant change from the control value ($p = .95$).

RESULTS

The means and standard deviations of the blood constituents before and after stress are given in Table 1. To facilitate evaluation of the changes, the results are shown graphically in Figure 1. The temperature fell and remained low during the first two hours (Fig. 1-A). The only significant change in the heart rate occurred at the second hour, at which time it was lower than prior to stress. The systolic and diastolic pressures rose immediately after stress, had fallen significantly below the pre-stress values at the second hour, and remained lower (Fig. 1-B) during the period of observation. The changes in the total number of white cells

were not significant (Fig. 1-C). There was a significant increase in the number of neutrophils immediately after stress and at the second and fourth hours. The lymphocytes and the eosinophils were significantly decreased at the second hour after stress and the eosinophils remained low at the fourth hour (Fig. 1-C). The total proteins (Fig. 1-D) rose significantly immediately after stress, decreased at the first hour and re-

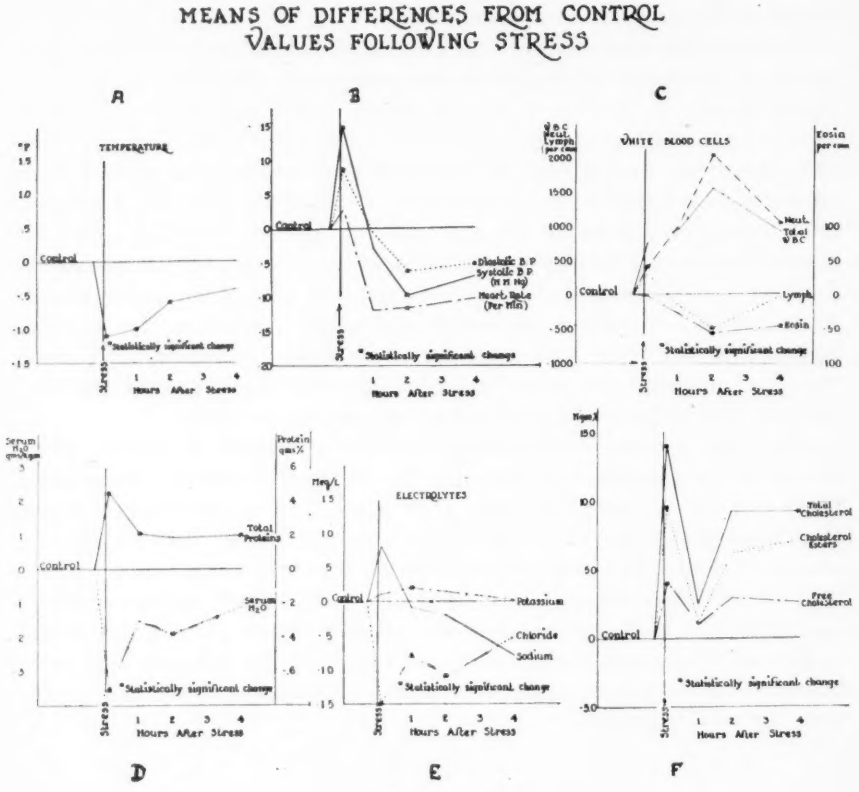

FIGURE 1

mained at this level for the duration of the observations. There was a corresponding decrease in the serum water concentration immediately after stress; this rose after two hours but was still significantly lower than the pre-stress value. Of the electrolytes (Fig. 1-E), the most significant changes occurred in the chloride levels, which decreased immediately after stress, rose slightly at the first hour and then decreased again. No significant changes were noted in the concentration of serum sodium, although it did

rise immediately after stress. There was a significant increase in the level of plasma potassium at the first hour. The levels of total cholesterol, cholesterol esters and free cholesterol rose immediately after stress (Fig. 1-F). Although the values fell after the first hour, the changes were not significant. There was, however, a significant increase in the total cholesterol at the fourth hour. Blood sugar and ascorbic acid levels did not show significant changes and were therefore not charted.

The changes in the urine constituents which occurred following stress are presented in Table 2 and graphically in Figure 2. There was a sig-

TABLE 2. MEANS AND STANDARD DEVIATIONS OF VALUES FOR URINE CONSTITUENTS BEFORE AND AFTER STRESS

	Number of subjects	Control		Immediately after stress		1 Hour after stress		2 Hours after stress		4 Hours after stress	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Sodium (mEq./hr.)	12	6.72	5.85	10.79	7.21	15.29	13.84	15.16	13.08	10.91	9.23
Potassium (mEq./hr.)	12	3.4	2.19	4.83	3.29	6.03	5.47	6.72	6.59	6.96	5.79
Chloride (mEq./hr.)	12	8.75	8.73	9.64	3.66	15.34	13.07	13.21	8.03	9.05	6.43
Ascorbic acid (mg./hr.)	11	.58	.40	1.04	1.19	1.81	1.88	1.11	.94	.72	.67
Uric acid (mg./hr.)	12	33.3	13.2	50.6	28.2	50.9	32.5	49.3	16.6	33.2	8.8
Creatinine (mg./hr.)	12	76.5	16.7	92.5	29.8	96.9	29.2	83.9	15.2	78.9	15.9
Uric acid/Creatinine	19	.39	.13	.52	.30	.48	.18	.56	.15	.42	.11

nificant rise in the excretion of ascorbic acid (Fig. 2-A). The only significant change which occurred in the excretion of electrolytes was in the sodium excretion, which increased immediately after stress (Fig. 2-B). The uric acid/creatinine ratio also increased at the second hour after stress (Fig. 2-D).

The 17-ketosteroids were determined in the urines of 6 subjects. The excretions, expressed as the percentage of the overnight urine specimens, are shown in Figure 3. These are compared to the data of Pincus and Hoagland (12) on 7 normal subjects. The means of the values in our subjects are reasonably close to those reported by Pincus. There was, however, considerable variation in the individual subjects. Therefore, on 4 normal subjects, determinations were made on a day when they were not stressed and samples of urine were collected at times identical to those of the stress day. The pattern of the excretion of ketosteroids in these normal subjects on a "no-stress" day was similar to those reported by other observers (12, 13). Again, however, the absolute amounts excreted varied considerably in each individual. Following the stress, there was an increased excretion of ketosteroids over the non-stress day in SL (Fig. 4), and a slight decrease in PL, DA and BR.

DISCUSSION

In the selection of determinations directed at identifying the response of the adrenal cortex to stress, one is faced with the fact that each determina-

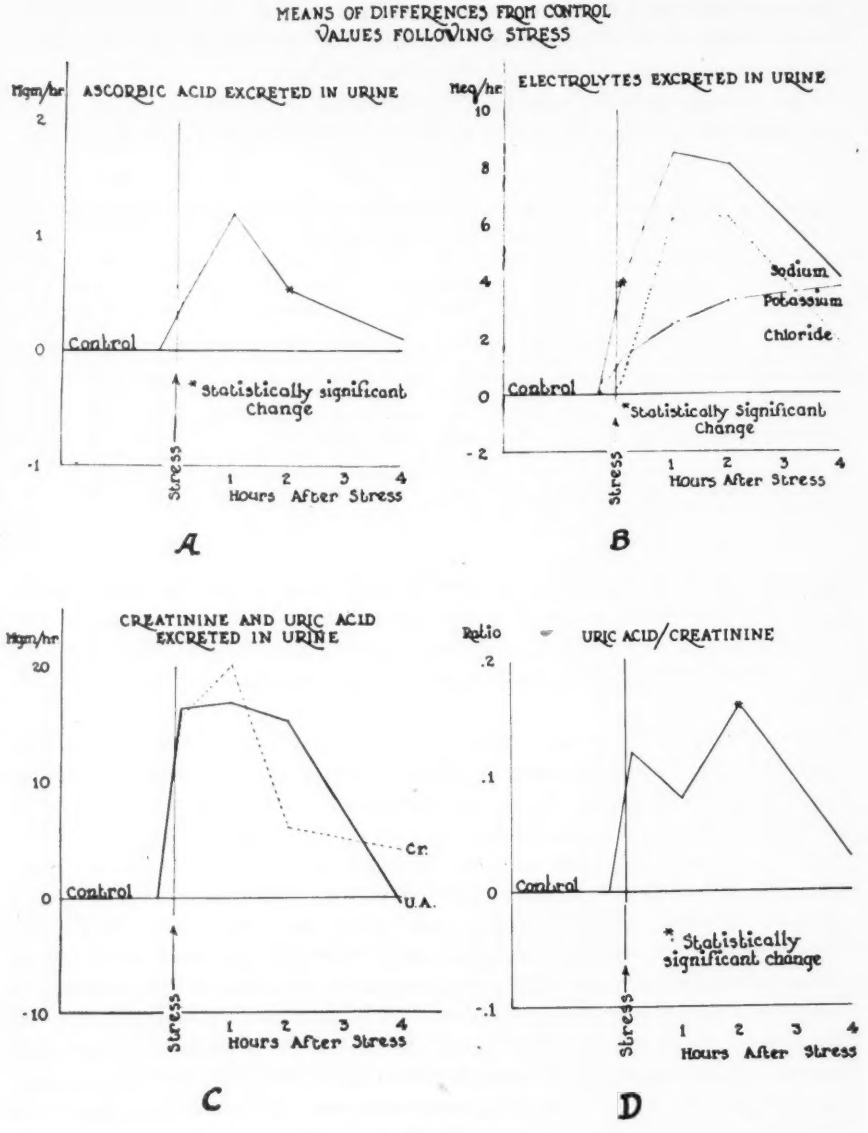

FIGURE 2

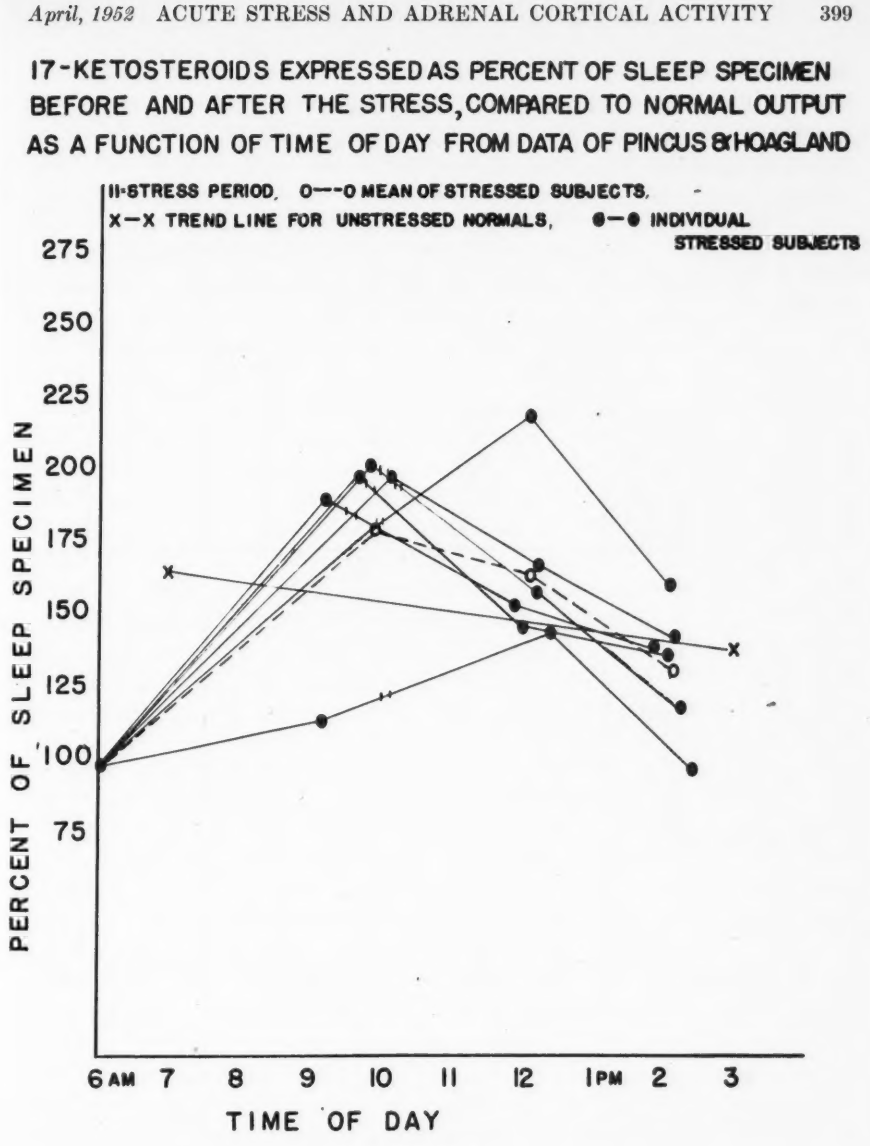

FIGURE 3

tion indicative of adrenal cortical activity may also be influenced by other physiologic factors. In the experimental animal, the decreases in the levels of adrenal ascorbic acid and of adrenal cholesterol are reasonably clear indications of adrenal cortical activity. In the human subject, such deter-

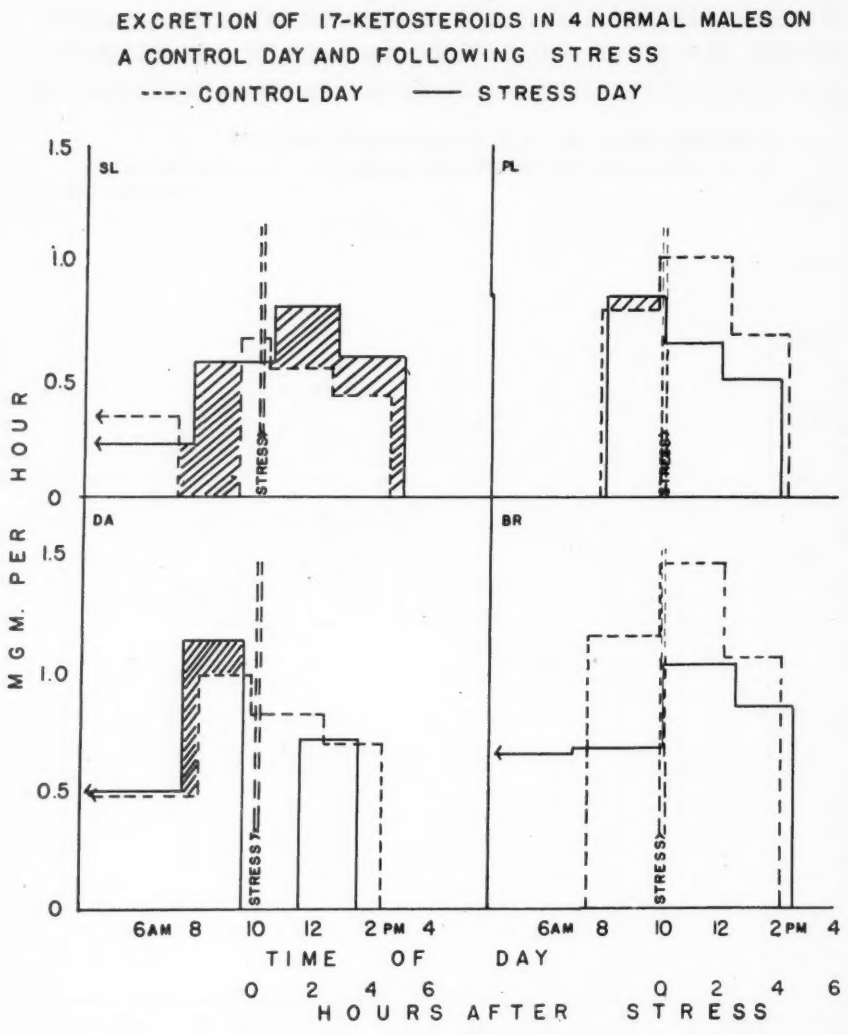

FIGURE 4

minations are not possible. It is, therefore, necessary to perform a series of determinations indicative of adrenal cortical activity and to evaluate the elicited responses. We selected determinations the results of which, on the basis of previous experimental and clinical data, have been reported as indices of adrenal cortical function (14). An additional problem was posed in this study by the nature of the stress. Obviously, immersion in

cold water will induce circulatory changes and it was, therefore, important to determine at what time following the stress, the circulatory changes had become stabilized. Examination of the data led us to the conclusion that after the first hour, the circulatory changes were not a factor in influencing the serum constituents, and that the various significant changes that occurred in the results from the second hour on, were indicative of adrenal cortical function.

There is considerable evidence that the levels of lymphocytes and eosinophils reflect adrenal cortical activity. Some of this evidence has been derived from studies on the effects of injected ACTH and cortisone (15, 16, 17, 18). Other data have been obtained from studies in the adrenalectomized animal (16, 19). Both ACTH and cortisone cause a lymphopenia and eosinopenia in the intact animal and in the normal subject. Adrenalectomy on the other hand, in both rats and mice, is associated with a lymphocytosis and an increase in the number of eosinophils. In the human subjects in our experiments, a significant lymphopenia occurred at the second hour, suggestive of an increase in the output of the adrenal cortical hormones. There was also a significant eosinopenia in our subjects at the second hour after stress. This eosinopenia persisted until the end of the experiment, and may also be considered as an indication of increased adrenal cortical activity. As far as the neutrophils are concerned, although they apparently are not under the exclusive control of the adrenal cortex, a consistent neutrophilia has been observed experimentally following injection of ACTH. Palmer, Cartwright and Winthrop (20) observed a consistent neutrophilia in swine following the injection of ACTH. In the human subjects in our experiments, there was a significant increase in the neutrophils at the second hour and at the fourth hour, which may have been due to stimulation of the pituitary adrenal mechanism. The changes in the total number of white blood cells in these subjects were not significant, though the counts did rise following the stress.

The circulatory changes immediately following the stress appeared to have influenced the distribution of body water, as reflected in the changes in the levels of serum water and total proteins at this time. The value for serum water fell significantly and there was a corresponding increase in the value for total proteins. By the end of the first hour, the total proteins had decreased but were still above the control levels and the serum water had increased but was still below the control level.

The importance of adrenal cortical hormones in the maintenance of normal serum levels of sodium and potassium is generally acknowledged. However, in acute experiments associated with changes in hemodynamics and hemoconcentration, it is difficult to attribute the changes in serum electrolytes solely to alterations in adrenal cortical function. Furthermore,

in a short-term experiment, the effects of adrenal cortical hormones on the level of serum electrolytes might not be evident. Mason *et al.* (21) found that electrolyte metabolism was essentially undisturbed by the administration of 100 mg. of ACTH, as judged by the concentrations of sodium, potassium and chloride in the blood and urine. In our experiments, a significant decrease occurred in the level of serum chloride at the first and second hours after the stress, paralleling the changes in the serum water. The increase in the level of plasma potassium at the first hour after stress is probably due to increased muscular activity.

Experimentally, the concentration of adrenal cholesterol decreases following stress or ACTH. The question arises as to whether or not the level of serum cholesterol reflects a change in the level of adrenal cholesterol. In human subjects treated with ACTH, Conn has reported (22) that after three to five days there is a decrease in the level of total cholesterol, largely in the ester fraction. Cortisone administration in normal subjects, according to Thorn (23), has no consistent effect on the level of serum cholesterol. Wolfson (24) has reported an increase in the concentration of serum cholesterol, largely in the ester fraction, following therapy with cortisone. The effects of the stress used in our experiments were probably of too short duration to cause any significant changes in the serum cholesterol level. Immediately after the stress, the values for total cholesterol, cholesterol esters and free cholesterol increased. At the first hour, there was a sharp decrease from these levels, which was not statistically significant. At the end of the experiments, the value for total cholesterol was significantly above the control value. The increase was largely due to an increase in the esters, although the latter was not statistically significant. Although the increase in the value for total cholesterol at the fourth hour might be interpreted as reflecting adrenal cortical activity, one hesitates to draw any final conclusion in such a short-term experiment.

The results of the urine analyses are reported for only 12 of the 21 subjects. The discrepancy is due to the fact that the control specimens in the other 9 subjects were not timed accurately enough by the subjects. Sodium excretion was significantly increased immediately following the immersion, probably reflecting alterations in hemodynamics and kidney function due to the sudden immersion in cold water. None of the other changes in the electrolyte excretion was significant, although suggestive. For example, sodium excretion continued to increase and then fell to the control level. The chloride excretion paralleled the sodium excretion. The potassium excretion increased steadily during the course of the experiment.

An increase in the uric acid/creatinine ratio in the urine has been stated

by some observers (17) to be an indication of adrenal activity. A significant increase in this ratio occurred two hours following the stress in our subjects and may be a reflection of adrenal cortical activity.

Excretion of ascorbic acid is particularly interesting because of the importance of ascorbic acid to the adrenal cortex and also because, experimentally, the level of adrenal ascorbic acid decreases following stress or ACTH. Beck, Browne and MacKenzie have recently reported (25) an increased excretion of ascorbic acid following injection of ACTH into human subjects. The degree to which the ascorbic acid excretion increased depended on the intake of ascorbic acid. Cortisone had no effect on ascorbic acid excretion. In our experiments, there was an increase in the excretion of ascorbic acid in the urine at the first hour and a significant increase at the second hour. During these periods, there were only small changes in the blood levels. Variations in the excretion of ascorbic acid, however, could readily occur within the limits of the blood levels observed. It is impossible to say whether or not the increased excretion of ascorbic acid is indicative of increased adrenal cortical activity. It may be the result of the increased rate of glomerular filtration of ascorbic acid. The latter is known to occur following injections of ACTH and may also occur following the associated hemodynamic changes consequent to stress (26).

The 17-ketosteroid excretion in these subjects followed the daily pattern observed by other investigators (27). The excretion varied in each individual and, from the data available, it would seem that over the period of this short stress, the excretion of 17-ketosteroids was not significantly different from the excretion in the same subjects on a control day.

The experimental data obtained in these experiments following the period of immersion in cold water provide a pattern of the reaction of normal male subjects to stress of short duration. The difficulty of measuring the degree of adrenal cortical activity is obvious, but it seemed to us that the significant and consistent eosinopenia and the associated significant increase in the uric acid/creatinine ratio might be taken as measures of adrenal cortical activity. The lymphocytopenia and the increased excretion of ascorbic acid may also reflect adrenal cortical activity. It is important to realize that the changes that occurred represent the combined effects of both psychologic and physiologic stimuli. This is particularly true of the fall in eosinophils. Thorn (28) reported an eosinopenia, not only in the members of the Harvard crew, but in the coach and coxswain as well. Furthermore, on the day of the competitive race when the emotional tension was at the peak, the eosinopenia was greatest, although the physical stress was approximately the same.

SUMMARY

Twenty-one normal males were immersed in water at 9.5°C. for a period of eight minutes. The acute changes were followed by determinations on blood and urine samples taken prior to the immersion, immediately following and at one, two and four hours after the immersion. The statistically significant changes which occurred and the time of their occurrence after immersion were as follows:

1. Decrease in temperature (immediate, 1–2 hours).
2. Increase in systolic and diastolic blood pressure (immediate) and decrease (2 hours).
3. Decrease in heart rate (1, 2 and 4 hours).
4. Increase in neutrophils (immediate, 2 and 4 hours).
5. Decrease in lymphocytes (2 hours).
6. Decrease in eosinophils (2 and 4 hours).
7. Decrease in serum chloride concentration (immediate, 1 and 2 hours).
8. Increase in total protein concentration (immediate, 1 and 4 hours).
9. Decrease in serum water concentration (immediate and 2 hours).
10. Increase in total cholesterol concentration (immediate and 4 hours), associated with an increase in the level of cholesterol esters and free cholesterol (immediate).

The significant changes in the urinary constituents were:

1. Increased excretion of ascorbic acid (2 hours).
2. Increased excretion of sodium (immediately following the stress).
3. Significant increase in the uric acid/creatinine ratio (at the second hour). The levels of serum sodium, plasma potassium, blood sugar, blood ascorbic acid, and potassium and chloride in the urine were also followed, but statistically significant changes were not observed in these constituents.

The suggestion is made that the changes in the lymphocytes, eosinophils and uric acid/creatinine ratio are indicative of adrenal cortical activity.

Acknowledgment

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The statistical analysis was made by Miss Elizabeth Street.

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THE EFFECT OF CORTISONE ON THE IODINE ACCUMULATING FUNCTION OF THE THYROID GLAND IN EUTHYROID SUBJECTS*

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THIRTY years ago Marine and Baumann (1) noted that adrenal insufficiency in rabbits led to an increase in heat production and respiratory exchange and to the appearance of a symptom complex resembling exophthalmic goiter. Marine (2) subsequently reviewed the evidence pointing to the importance of a primary adreno-gonadal insufficiency as a possible fundamental cause of Graves' disease. The occurrence of Graves' disease following roentgen ray damage to the adrenal cortex in 2 cases reported by Oppenheimer (3) provided a direct clinical example in support of this concept. The pathologic changes observed in patients who have died of thyrotoxicosis—enlargement of the thymus, diffuse lymphoid hyperplasia and small adrenal cortices—have been cited as evidence suggestive of adrenal cortical insufficiency (2).

The endocrine antithesis to the hyperthyroid-hypoadrenocortical state is well demonstrated by the frequent occurrence of an atrophied thyroid gland in Cushing's syndrome (4). The high concentration of serum cholesterol, the dry skin, and the low BMR frequently present in this disease (5) are clinically suggestive of hypothyroidism.

The recent availability of ACTH and cortisone has stimulated further experimental inquiry into the nature of thyroid-adrenal relationships. In Addison's disease, the thyroid depression following initial stimulation produced by cortisone therapy of more than four days' duration (6) led to a trial of ACTH therapy in hyperthyroidism, with variable results. Hill *et al.* (6) obtained clinical improvement in 3 patients and a decrease in the size of the thyroid in 6 thyrotoxic patients treated with ACTH over a 5-day to 23-day period.

Wolfson *et al.* (7) reported a detectable degree of hypothyroidism in all of 7 patients treated with ACTH or cortisone for prolonged periods. Hill *et al.* (6) also studied the effects on the thyroid of ACTH in 10 sub-

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jects, and of cortisone in 4 subjects with normal thyroid and adrenal function. These workers noted a fall in the I^{131} accumulation gradient, although the 24-hour iodine uptake did not change. They concluded that ACTH and cortisone did not inhibit but merely retarded I^{131} accumulation by the thyroid gland. In 8 of 13 patients serum protein-bound iodine levels fell but the BMR did not decrease until the changes in accumulation gradient and in the serum protein-bound iodine level became marked. Werner (8, 9) likewise failed to observe a decrease in the 24-hour uptake of I^{131} following ACTH therapy of toxic goiter. In a previous study (10, 11) we noted a fall in the 24-hour uptake of I^{131} from pretreatment normal values to hypothyroid levels in 2 patients with Hodgkin's disease treated with cortisone. In 3 others of the 6 patients studied there was a significant, though less marked, fall.

In view of the contradictory reports relative to the effect of ACTH and cortisone on the iodine accumulating function of the thyroid gland, as measured by the accumulation gradient on one hand and the 24-hour uptake on the other, further inquiry seemed indicated. Recent studies (12, 13) have shown that the rate of I^{131} clearance from the blood is probably the most reliable index of the iodine accumulating function of the gland. Because of the nature of the relationship between thyroidal and renal plasma clearances, significant changes in thyroid function may at times be observed in the presence of minor or equivocal changes in the 24-hour thyroidal uptake values. Conversely, changes in the 24-hour uptake may be spurious in that they may be related to alterations in renal function rather than to primary changes in the level of thyroid function. In the present study, investigations have been extended to include the effect of cortisone on the thyroidal and renal plasma I^{131} clearance rates as well as on the 24-hour uptake levels as determined by serial observations before, during and after cortisone therapy in various disease states not known to affect thyroid function.

METHODS

Subjects were hospitalized patients of the Veterans Administration Hospital, Bronx, N. Y. Those with known thyroid disease or other endocrine dysfunction and those who had received iodine in any form during the study were excluded.

Methods of assay of radioactivity in the thyroid and urine and the physiologic significance of thyroidal and renal plasma I^{131} clearance rates are being reported elsewhere (14). The thyroidal plasma I^{131} clearance is defined as the volume of plasma cleared of I^{131} per minute by the thyroid or, expressed mathematically, as the thyroid uptake over any time interval divided by the average plasma concentration during that

time interval. From an observed constant relationship between the body weight and the space of I^{131} distribution during the half-hour period following intravenous administration, it has been demonstrated that the clearance rates, accurate to within 10 per cent, may be obtained from the following formulas without the necessity for obtaining assays of plasma radioactivity (14):

$$Cl_{\text{thyroidal}}\ \text{ml./min.} = \frac{\text{half-hour thyroid uptake}}{\text{av. dose retained} \times 30} \times 20\%\ \text{body weight}_{\text{Gm.}} \quad \text{I(a)}$$

$$Cl_{\text{renal}}\ \text{ml./min.} = \frac{\text{half-hour renal excretion}}{\text{av. dose retained} \times 30} \times 20\%\ \text{body weight}_{\text{Gm.}} \quad \text{I(b)}$$

Where

$$\text{av. dose retained} = \text{admin. dose} - \tfrac{1}{2}(\text{half-hour thyroidal uptake} + \text{half-hour renal excretion}).$$

To obtain the half-hour thyroidal uptake, allowing for changes in the level of extrathyroidal activity during this period of time, the following formula is used (the derivation has been considered previously (14)):

$$\text{Thyroidal uptake} = \frac{OD + EU}{D - E}$$

Where D = dose injected in μc of I^{131}

O = observed increase in activity over the neck in μc of I^{131} during the first thirty minutes

U = μc of I^{131} excreted in the urine during the first thirty minutes

E = initial extrathyroidal activity in the neck in μc of I^{131} less the background value prior to injection

To obtain O and E, minute-by-minute readings of activity over the thyroid are recorded automatically by a Streeter-Amet printing register for thirty-three minutes following the injection of the isotope. The second-minute count is taken as the initial extrathyroidal activity (E) after the background has been subtracted. The total increase in counting rate from the second to the thirty-first minute is taken as the observed increase in activity (O).

In the determination of the clearance rates, the precision of repeated observations in the same individual is even greater than the 10 per cent estimated, since the variation from subject to subject is dependent chiefly on a variable space of distribution of the iodide over the half-hour period following the injection. Large increases in extracellular fluid increase the space of iodide distribution and thus give falsely low values for both thyroidal and renal clearance rates when calculated from formulae

I(a) and I(b). Therefore patients in whom obvious edema developed were excluded from further investigation. Several of the patients made small gains in weight up to 3 or 4 pounds over a period of two weeks or more, but this could be attributed to increase in appetite. Iodide space measurements before and during cortisone therapy were performed in Case 8 and found to be identical.

In this laboratory the 24-hour I^{131} thyroidal uptake measurements in euthyroid subjects range from 10 to 50 per cent. Values below 10 per cent are characteristic of the hypofunctioning gland. The range of thyroidal clearance measurements in euthyroid subjects is from 8 to 40 ml./min./1.73 M^2 surface area. Basal metabolic rates and assays of total serum cholesterol concentration (method of Schoenheimer-Sperry (15)) were performed in the clinical diagnostic laboratory.

RESULTS

Diagnoses, treatment schedules, thyroidal and renal clearance data, 24-hour uptake values, BMR's and serum cholesterol values for all patients studied are given in Table 1. The effect of cortisone on the thyroidal and renal plasma I^{131} clearance rates and on the fraction of the dose of I^{131} taken up by the thyroid at the end of twenty-four hours is summarized in Figures 1, 2, and 3.

A. *Changes in thyroid function.*

Group I. Cases 1–18 (19 studies in 18 patients). All of these patients showed significant and often very marked falls in thyroidal I^{131} clearance and/or 24-hour uptake values shortly after institution of cortisone therapy. This was noted as early as the fifth day (Case 10), sixth day (Case 11) or eleventh day (Case 3) of treatment with large doses of cortisone (300 mg. on the first day followed by 200 mg. daily). In most cases re-evaluation was not obtained until after about two weeks of treatment, at which time depression of iodine accumulation by the thyroid was evident in all cases in this group.

In 4 patients (Cases 3, 10, 13 and 16) thyroidal clearance rates and 24-hour uptake measurements during therapy were in the hypothyroid range. Two patients (Cases 2 and 15), whose clearance rates were not obtained, had 24-hour uptake values in that range. Two patients (Cases 13b and 9), on whom 24-hour studies were not performed, had clearance rates in the hypothyroid range. Thus 8 of the 19 studies showed a decline from pretreatment normal values to hypothyroid levels under therapy. In several other patients, borderline values almost as low were observed.

In those instances in which multiple observations were obtained during the course of therapy, a progressively increasing inhibition of iodide

TABLE 1

Case	Diagnosis	Cortisone		I^{131} Tracer studies					BMR, %		Serum cholesterol, mg./100 cc.	
					Clearance values (ml./min./1.73M²)		24-Hour values (% of dose)					
		Date	Dosage, mg./day	Date	Thyroid	Renal	Thyroid	Urine	Date	Value	Date	Value
1	Acute rheumatic fever	1950 8/23 8/24 to 9/12 9/13, 14 9/20 to 22	200 100 0 100	8/22* 9/12 11/15† 12/4†			34* 19 28† 29†	50* 77 58† 60†				
2	Vernal conjunctivitis	1950 9/5 to 21 9/22 to 30 10/1 to 5	100 200 100	8/23* 10/4 11/6†			27* 7 26†	56* 91 54†				
3	Iridocyclitis	1950 11/3 11/4 to 10 11/11 to 15, 17	300 200 100	11/1* 11/14 11/27†	13* 1 10†	31* 58 35†	33* 10 26†	62* 80 65†	11/15 11/27†	−16 − 2†	11/10 11/17	163 151
4	Acute iritis	1950 10/16 to 10/25 10/26 to 11/3, 5 11/7, 9	200 100 50	10/11* 11/1 11/13†	20* 8 22†	35* 65 48†	37* 14 41†	43*‡ 90 58†	11/8	−13	11/10	212
5	Anterior and posterior uveitis	1950 12/14 to 27	200	12/11* 12/28 1/18/51	19* 8 26†	54* 64 41†	40* 18 32†	48* 75 52†	12/2* 1/3/51†	+ 4* −10†	12/8* 12/15 12/29	222* 220 139
6	Central retinopathy	1951 3/16 3/17 3/18, 19 3/20 to 4/10	200 300 200 100	3/15* 4/3 4/23†	15* 8 unreliable§	26* 64 30†	22* 13	63* 58	3/15* 3/20 3/26	−14* +33 + 7	3/14* 3/16 3/23 3/30 4/6	261* 260 204 232 270
7	Scleroderma	1950 11/8 to 10 11/11 to 12/4 12/5,7,9,11,13, 15,17,19,22, 25,26 and 1/1,4, 1951	200 100 100	10/13* 11/21 1/8/51†	31* 15 31†	26* 39 23†	47* 26 48†	49* 63 50†				
8	Iridocyclitis	1950 12/6 12/7 to 17 12/18 to 26 12/28,31 1/2, 1951	300 200 100 200 100	12/5* 12/19 1/2/51 1/22†	26* 18 18 27†	40* 60 53 51†	38* 20 21 39†	49* 71 45‡ 51†	12/5* 12/14 1/4/51	+ 8* −14 −22	12/8 12/29	158 247
9	Sympathetic ophthalmitis	1951 5/31 6/1 6/2 to 20	300 200 100	5/28 6/21 7/31†	18* 5 16†	41* 46 38†	28* 28†	68*	5/29* 6/11	+12* − 4	6/6 6/15	211 248
10	Rheumatoid arthritis; uveitis	1950 11/22 11/23 11/24 to 12/14 12/15 to 18 12/23 12/24 to 1/2, 1951 1/3 to 9 1/10 to 13	300 200 100 50 100 200 100 50	11/14* 11/27 12/13 1/4/51 1/15†	32* 9 9 0 23†	24* 36 40 42 21†	42* 21 18 9 34†	54* 65 83 82 55†	11/30* 12/11 1/4/51 1/10	+20* +20 +28 +11	11/24 12/1 12/8 12/15 12/29 1/12/51	196 204 181 181 185 174
11	Kaposi's sarcoma	1950 11/28 11/29 to 12/7 12/8 to 12/28 1951 1/4, 1/8,11, 15,18,	300 200 100 100	11/21* 12/4 12/19 1/17/51 7/25†	14* 9 11 8 13†	38* 77 59 32 44†	37* 20 19 24 31†	59* 81 62 61	12/6 12/19 12/26 1/2/51 1/16 1/17 1/24†	− 4 − 1 − 9 − 4 −22 − 7 −15†	11/27* 12/4 12/11 12/18 12/27 1/3/51 1/8/51 1/15 1/22†	244* 202 164 250 155 145 196 200 200†

TABLE 2—*continued*

Case	Diagnosis	Cortisone		I^{131} Tracer studies					BMR, %		Serum cholesterol, mg./100 cc.	
				Date	Clearance values (ml./min./1.73M²)		24-Hour values (% of dose)					
		Date	Dosage, mg./day		Thyroid	Renal	Thyroid	Urine	Date	Value	Date	Value
12	Corneal infiltrate	1951 4/24 4/25 4/26 to 5/17	 300 200 100	4/23* 5/10 8/8†	10* 1 8†	35* 32 28†	34* 8 30†	51* 62			4/23* 5/4 5/11 5/18†	209* 220 294 178†
13	Rheumatoid arthritis; iritis	1950 10/20 to 11/4 11/5,6,8 1951 6/1 to 4 6/5 to 8 6/9 to 10 6/11 to 18	 200 100 200 100 200 100	10/19* 11/8 11/16† 5/31/51*† 6/18	22* 12 23*† 5	41* 47 60*† 52	46* 28 47*† 12	54* 59 65† 21*† 37			11/10	188
14	Bilateral iridocyclitis	1950 11/9 11/10 11/11 to 15,17, 19,21 11/30 12/1 to 6 12/7 to 12 12/26 12/27 to 30 12/31 1951 1/3 to 5,7 1/9,11	 300 200 100 300 200 100 300 200 300 100 50	11/6* 11/21 12/7 12/28 1/18/51† 3/8†	38* 13 14 16 7† 28†	48* 61 81 59 41† 47†	41* 23 21 23 19†	60* 70 79 72 60†	11/14 12/4 12/13 12/21 1/3/51 1/10	+5 −11 +2 −6 −1 −10	11/6* 11/10 11/17 11/24 12/1 12/8 12/15 12/29 1/4/51 1/12	88* 187 134 200 178 162 129 128 195 126
15	Pemphigus vulgaris	1950 10/27 10/28 to 30, 11/2,4,6,8 11/10 to 14 11/16 to 18 11/19 to 22 11/23,24 11/25,26 11/27 to 29 11/30 to 12/2 12/3 to 7 12/8,9 12/10 to 14 12/15 to 17 12/19 to 25 12/26 to 28 1951 1/4,5 1/6 to 10 1/11 to 21	 300 200 100 200 100 75 300 200 300 200 150 100 75 50 25 100 200 100	10/17* 11/8 11/27 12/11 1/11/51	 10	 52	37* 12 9 4 20	57* 78 85 81 68	12/7 12/19 12/26 1/2/51 1/9 1/16 1/23 2/6	+4 +14 −10 −9 −5 −4 −8 −4	11/10 11/27 12/4 12/11 12/18 12/23 1/3/51 1/8 1/15	271 244 250 194 216 204 266 291 262
16	Keratitis, parenchymatous	1950 12/8 12/9 to 22 12/26 to 28 12/29 1951 1/1,2	 300 200 200 175 150	11/20* 12/4* 1/3/51	23* 2	46* 78	39* 36* 3	55* 57* 63	11/29* 12/12	−1* −4	11/17* 11/24 12/1 12/8 12/15 1/4/51	252* 264 220 206 216 196
17	Uveitis, chronic	1950 10/2 to 23	 200	9/26* 10/23			27* 11	55* 68				
18	Behcet's syndrome	1950 10/20,21 10/22–23,26 10/29 10/30 10/31 11/6 11/7,8 11/9	 200 100 300 200 100 300 200 100	8/14* 11/9			22* 14	64* 77				

TABLE 1—*continued*

Case	Diagnosis	Cortisone		I^{131} Tracer studies					BMR, %		Serum cholesterol, mg./100 cc.	
				Date	Clearance values (ml./min./1.73M²)		24-Hour values (% of dose)					
		Date	Dosage, mg./day		Thyroid	Renal	Thyroid	Urine	Date	Value	Date	Value
19	Bilateral uveitis	1951 2/19 2/20 2/21 2/22 to 26 2/27 to 3/2, 4,6	 100 300 200 100 50	2/14* 3/5	24* 21	49* 45	40* 33	56* 45	2/12* 2/26	− 1* − 4	2/16* 2/23 3/2	254* 267 226
20	Exfoliative dermatitis	1950 12/11, 12 12/13 to 18 12/29,30 12/31 to 1/2 1951 1/3 to 6 1/12 to 21 1/22 to 26 1/29 to 2/1 2/2 to 12 2/13 to 22, 24, 27	 200 100 200 100 50 100 50 25 50 25	12/7* 1/11/51	15* 8	46* 36	23* 22	39*‡ 57	1/3 1/10 1/17 1/24	+19 − 2 + 8 − 1	12/6* 12/18 1/3 1/5 1/8 1/15 1/22 1/29	158* 234 244 243 270 240 263 249
21	Bilateral uveitis	1951 1/18 1/19 to 22 1/23 to 25 1/26 1/29 to 2/2 2/3 to 8	 300 200 100 200 100 200	1/16* 2/1	12* 17	34* 60	23* 23	55* 66	1/16* 1/25 2/8	− 5* + 4 + 1	1/17* 1/19 1/26 2/2	221* 172 146 148
22	Acute iritis O.D.	1950 11/22 11/23 11/24 to 29 12/1 to 3	 300 200 100 100	11/21* 12/4	13* 12	30* 35	36* 36	45* 64	11/20* 11/22* 12/11†	+ 6* −20 − 7†	11/17* 11/24 12/8†	310* 280 262†
23	Sulzberger-Garbe's disease	1950 10/20 10/21 10/22,23,25, 27,29,31 11/1,3 11/9,12,15	 300 200 100 200 300	9/28* 11/9	37* 36	30* 38	39* 40	27*‡ 44			10/4* 11/10	212* 271
24	Keratitis dendritica	Subconjunctival injections (2.5 mg.) on 3/12,13,16, 20,23,26; 4/3,6		3/14* 3/28	13* 12	28* 25	32* 29	63* 64				

* Pre-cortisone study.
† Post-cortisone study.
‡ Incomplete urine collection.
§ Thyroid counter stopped functioning during test.

accumulation by the thyroid gland was observed (Cases 10 and 15), unless the dosage had been decreased (Case 11) or therapy temporarily interrupted (Case 14). Figure 4 illustrates the behavior in one of these patients (Case 10).

In 5 instances post-treatment investigations could not be performed. In all but 3 of the remaining studies, the post-treatment tests showed prompt return to or near pre-treatment control values. This was evident as early as the fourth day (Case 4) or the fifth day (Case 7) after cessation of therapy. In Case 10 this response was seen only two days after the drug was discontinued entirely but for three days prior to omission, only 50

mg. a day had been given. This patient had shown an extremely low thyroidal clearance rate and 24-hour uptake two weeks before.

Of the 3 cases in which there was failure to demonstrate a prompt return to control values, in 1 (Case 15) the post-cortisone study was not obtained until after one week's therapy on a second course; it showed a

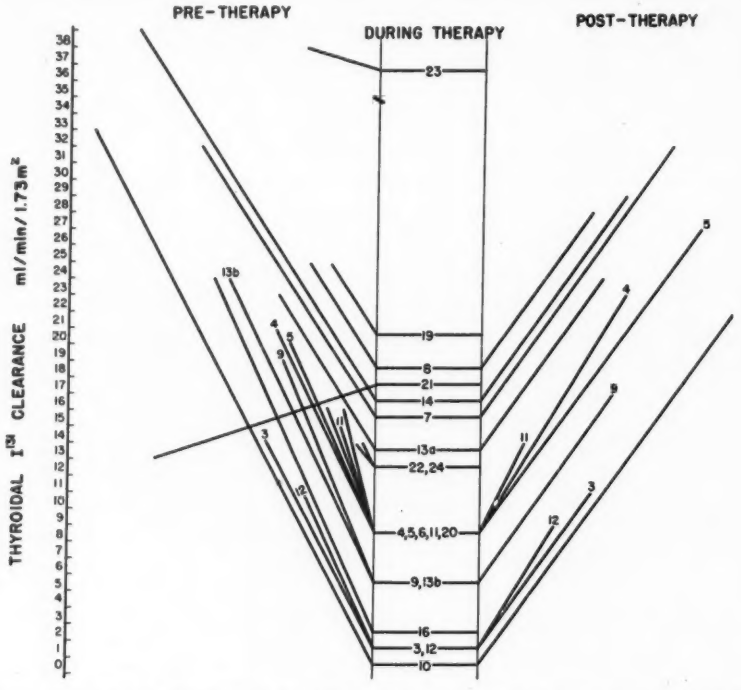

FIG. 1. The effect of cortisone on thyroidal plasma I^{131} clearance rates. When more than one study was made during therapy, only the result of the last study during therapy is plotted, because of the tendency to increased effect as treatment was prolonged.

value intermediate between the pre-treatment level and the low point of the preceding therapy. In the remaining 2 cases (Nos. 13 and 14) there was a definitely delayed return to normal.

Thus, although there was some variability in the speed with which return to normal function was effected, most patients demonstrated a rapid reversal of even markedly depressed function within a few days after the influence of cortisone was removed.

Group II. Cases 19 and 20. These subjects showed only an equivocal response. In Case 19 there was a slight fall in the thyroidal clearance rate

and in the 24-hour uptake values. It should be noted that at the time of the studies during therapy, this patient was receiving 50 mg. of cortisone every other day. In Case 20 there was a moderate drop in thyroidal clearance, although the 24-hour uptake value did not change. This patient

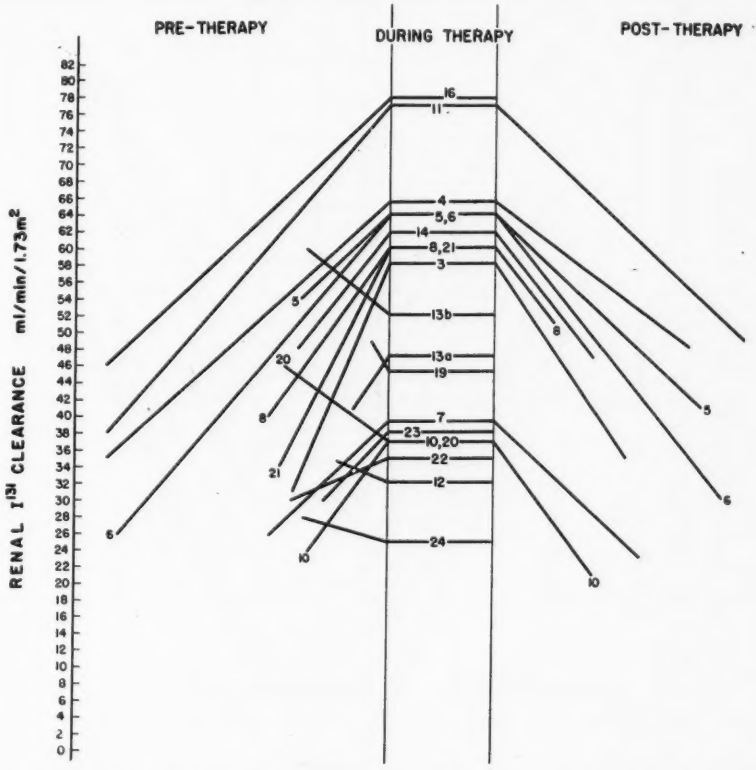

FIG. 2. The effect of cortisone on renal plasma I^{131} clearance rates. When more than one study was made during therapy, only the result of the first study during therapy is plotted, because of the tendency to regression towards pretreatment levels as treatment was prolonged.

had received no cortisone for four days prior to the test, although for three weeks before that, substantial doses had been administered daily.

Group III. Cases 21–24. In this group, inhibition of thyroidal iodine accumulation was not observed. Only in Case 21 can it be said that cortisone therapy failed to influence thyroid function. In Case 22 the patient was receiving only 100 mg. of cortisone every other day at the time of the study during therapy. In Case 23 no study during therapy was obtained,

the repeat test having been performed six days after the last dose of cortisone. In Case 24 the patient received only 4 doses (each 2.5 mg.) of subconjunctival cortisone between the two tests.

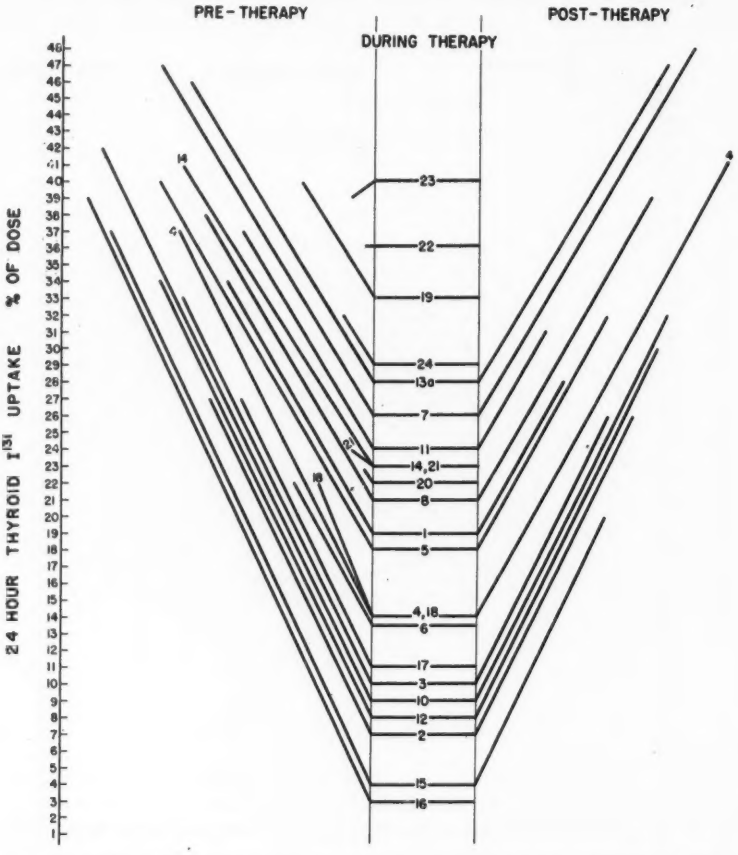

FIG. 3. The effect of cortisone on 24-hour I^{131} uptake by the thyroid gland. When more than one study was made during therapy, only the result of the last study during therapy is plotted, because of the tendency to increased effect as treatment was prolonged.

Basic metabolic rate and serum cholesterol concentration. In a few instances (Cases 3, 8 and 9) the BMR seemed to change in the same direction as the thyroidal iodine tests. However, at times, the changes in the opposite direction were just as marked, and readings in general were too variable for us to be convinced of their significance.

Consistent changes in total serum cholesterol levels were not observed.

B. *Changes in renal function.*

As may be seen from Figure 2, the rise in renal clearance during therapy was in many cases as striking as the fall in thyroidal clearance. In some instances, however, there was only an insignificant change in either direction. In 2 patients (Cases 11 and 14) the renal clearance during ther-

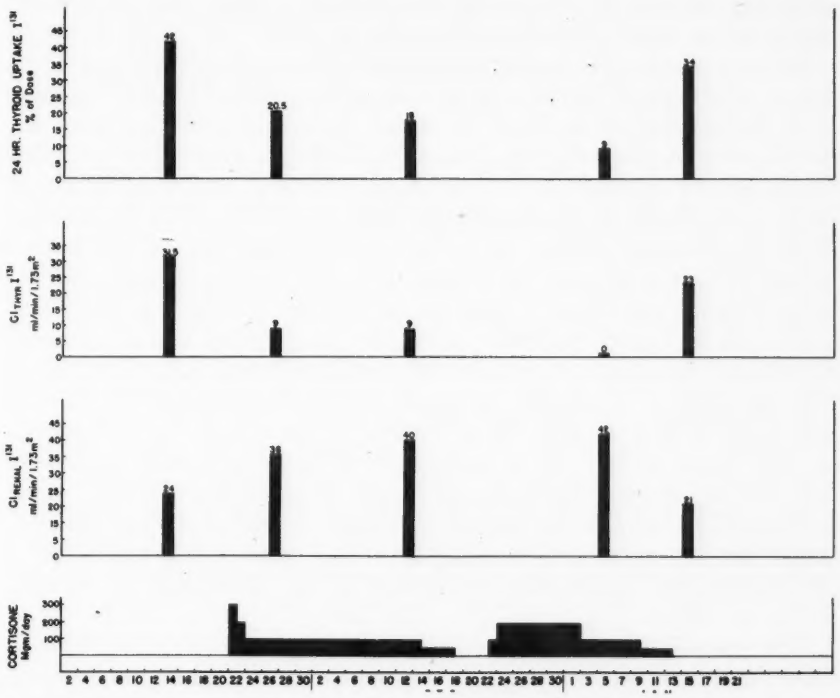

FIG. 4. Course of renal and thyroidal I^{131} clearances and 24-hour thyroidal uptake in Case 10 before, during and after cortisone therapy.

apy tended to return to pre-treatment levels, even while thyroid accumulation remained inhibited. This behavior was not evident in Case 10.

DISCUSSION

From the results presented, there appears to be little doubt that cortisone in adequate doses is capable of inhibiting markedly the accumulation of iodide by the normal thyroid gland. Although most of the investigations in this study were carried out over periods of only a few weeks, the evidence suggests that there is no "escape phenomenon" under continuous cortisone

therapy, at least for periods up to almost two months. In fact, an increasing effect was observed as the period of therapy lengthened, provided there was no interruption of treatment. The return to normal was as rapid as the initial depression and was evident, in most cases, within a few days following discontinuation of therapy. Although the study was not designed to evaluate the effect of various dosage levels, it appears from the available data that 100 mg. a day is effective, whereas 50 mg. a day is relatively ineffective in altering the state of thyroid function.

If it is accepted that iodide accumulation by the thyroid gland is correlated with thyroid hormone formation, as it must be in some manner, then it may be concluded that the output of thyroid hormone is kept at a low level by cortisone therapy. The studies of Hill *et al.* (6) showing clinical improvement with ACTH in hyperthyroid subjects, and those of Hardy *et al.* (16) demonstrating a depressing effect of ACTH on the level of serum PBI, confirm this point of view. The only occasional correlation of basal metabolic rate with iodine accumulating function of the thyroid observed in the present study may be due to two factors. Firstly, it must be conceded that the basal metabolic rate cannot be regarded as a quantitative measure of the effect of thyroid hormone, even though extremely high or low figures may be reliably indicative of thyroid disease. Secondly, at most, the BMR is a reflection of the level of circulating thyroid hormone and its effect on the body, rather than of the level of thyroid function at the time of the test. Thyroid hormone has a biologic life of several weeks at least. Therefore, even marked inhibition of new hormone formation would not be expected to be reflected in a definite clinical expression of hypothyroidism within the short period of the investigations reported here.

The mechanism of inhibition cannot be elucidated from these studies. A direct effect on the thyroid gland itself might lead secondarily to increased amounts of active circulating TSH, with resulting increase in the vascularity of the thyroid and perhaps the production of exophthalmos. No such changes resulting from cortisone therapy have been reported nor have we observed such phenomena. If the cortisone acts by inhibiting pituitary TSH production, as is very likely the case, this biologic experiment then serves to illustrate the nonspecific inhibition of an hypophysial tropic hormone by a hormonal substance presumably not controlled by that tropic hormone.

Soffer *et al.* (17) have shown that epinephrine leads to increased amounts of circulating thyrotropic factor. From subsequent studies (18) they concluded that the intact adrenal cortex as well as cortisone inhibited the TSH-stimulating effect of epinephrine. Further quantitative studies of circu-

lating and urinary TSH are necessary to aid in interpretation of the mechanism of inhibition by cortisone.

It is of interest that increased endogenous adrenal cortical hormonal activity leads to a similar effect on the thyroid. Selye (19) has noted thyroid atrophy early during the alarm reaction and Paschkis *et al.* (20) have shown an inhibition of I^{131} uptake by the thyroid of rats shortly after the alarming stimulus of intramuscular formalin injections. Signs and symptoms suggestive of the low thyroid function often accompanying Cushing's syndrome have already been commented upon.

The nature of the relationship of thyroidal plasma I^{131} clearance to the 24-hour uptake of I^{131} by the thyroid should be emphasized. The latter depends on a number of factors other than the thyroidal clearance rate, chief of which is the renal clearance of iodide. Alterations in renal function alone may give rise to changes in the 24-hour uptake by the thyroid. Thus increased renal clearance of iodide leads to depression of the 24-hour thyroidal uptake, even when thyroidal clearance remains unchanged, whereas the reverse is true when the renal clearance falls. For this reason the effect on thyroid function cannot be properly evaluated by measurement of the 24-hour uptake alone, unless it can be shown that the rate of renal clearance has not been affected.

In several of the cases reported here, the effect of cortisone on the thyroid as measured by the 24-hour uptake was falsely enhanced, owing to the increase in renal clearance of iodide. In general, the changes in 24-hour uptake (Fig. 3) appear more striking than those of thyroidal clearance (Fig. 1) for this reason. The thyroidal I^{131} clearance rate is an absolute measure of the iodide accumulating function of the thyroid gland, whereas the 24-hour uptake must be regarded as only a relative measurement.

The mechanism of increased renal clearance of iodide has not been investigated. There is the possibility that iodide is handled by the kidney in a somewhat similar fashion to chloride, although the renal clearance values obtained for I^{131} make it obvious that they are not treated quantitatively in an identical manner. The tendency for hypochloremia to develop as a result of cortisone therapy, and the ability of patients with Cushing's syndrome to excrete chloride in excess of normal with (21) or without DCA (22), are consistent with the view that the effects of the hormone on the kidney are to produce changes in chloride and iodide excretion in the same direction.

In view of the increase in renal clearance of iodide noted in this series, it is difficult to explain the findings of Hill *et al.* (6). These workers noted that under ACTH and cortisone therapy there was a decrease in the accumulation gradient during the first few hours after administration of

the tracer of I^{131} but observed that the 24-hour uptake values were unchanged. They interpreted this to indicate that although the process of iodide accumulation was slowed down, the fraction of the dose picked up by the thyroid was not affected. Unless the renal accumulation rates decreased proportionately, the reverse of which has been demonstrated here, it would appear that this interpretation is untenable. The possibility that a vast accumulation of edema fluid might in part explain such a paradoxical situation seems unlikely in view of the short courses of therapy to which their patients were subjected.

The studies reported here suggest the possibility that some of the clinical and metabolic alterations accompanying long-term cortisone therapy are a result of hypofunction of the thyroid gland and may be in part reversed by the administration of thyroid substance or of thyroxine. Studies along this line are in progress.

SUMMARY AND CONCLUSIONS

1. The effect of cortisone on thyroid function has been evaluated by studies of thyroidal and renal plasma I^{131} clearance rates and/or 24-hour thyroidal uptake and renal excretion measurements in 24 euthyroid subjects. Doses of 100 mg. a day or more, with but a single exception, regularly produced marked inhibition of the iodine accumulating function of the thyroid gland and usually produced an elevation of the renal plasma I^{131} clearance rate. In almost all cases, the return to pre-treatment levels was manifest within a few days to a week following discontinuation of the drug or reduction of dosage to 50 mg. a day or less. Under continued therapy the thyroid inhibition became progressively more marked but renal clearances tended to return towards pre-treatment levels in some cases.

In general, close correlation of the iodine accumulating function with the BMR and the serum cholesterol concentration was not observed. It has been pointed out that significant alterations in these latter measurements may not be observed during a short course of cortisone therapy. Because alterations in renal clearance may produce spurious changes in the 24-hour thyroidal uptake of I^{131}, the desirability of evaluating thyroid function by measurements of thyroidal plasma I^{131} clearance rates has been emphasized.

The thyroid inhibition produced by cortisone is thought to be due to pituitary inhibition but there is no convincing proof of this hypothesis.

2. The possibility that hypofunction of the thyroid gland is responsible for some of the undesirable effects of cortisone therapy has been suggested.

Acknowledgments

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THE HEREDITARY OCCURRENCE OF TESTES AND ABSENCE OF SEXUAL HAIR IN AMENORRHEIC WOMEN: A TYPE OF PSEUDO-HERMAPHRODITISM*

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HERMAPHRODITISM has intrigued man from time immemorial. Innumerable references to the subject have appeared in both medical and nonmedical literature. Although the great majority of such cases are quickly recognized by the bizarre anomalous features of the external genitalia, they are not easily classified. A particular type of intersexuality represented by the cases reported in this paper has, for the most part, been unsuspected on clinical examination, and the diagnosis has usually not been made until surgery has been performed, or the condition has been discovered at autopsy. We refer to a special type of male pseudohermaphroditism, with normal-appearing external female genitalia and a completely feminine habitus. Evidence of masculinity is lacking, despite the presence of male gonadal tissue. In this article we present our conception of adequate clinical criteria for a positive diagnosis.

There can be, of course, all gradations of male pseudohermaphroditism; it is seen in patients having entirely feminine external features, as well as in those with masculine features. However, we shall limit the discussion entirely to cases which we believe are representative of this syndrome and shall exclude from consideration all other forms of intersexuality. There are examples of pseudohermaphroditism in which the outward characteristics of femininity approach those seen in this particular syndrome, but these have not been included because of the varied external features, such as a hypertrophied penile clitoris, absence of breast development, and testes located in the labia majora or in a bifid scrotum.

The familial occurrence of this syndrome will be emphasized, with particular reference to our group of cases, since they represent the largest reported collection in any one family (Fig. 1). Although only 2 of the 6 cases in our series have been proved microscopically, it is our belief that

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all 6 members of this family present identical syndromes, for the following reasons:

1. Common features (to be elaborated later) were present in all 6 subjects.
2. In the 2 patients requiring surgery, the operative findings were correctly anticipated.
3. All of the authenticated cases found in the literature disclose similar characteristics.

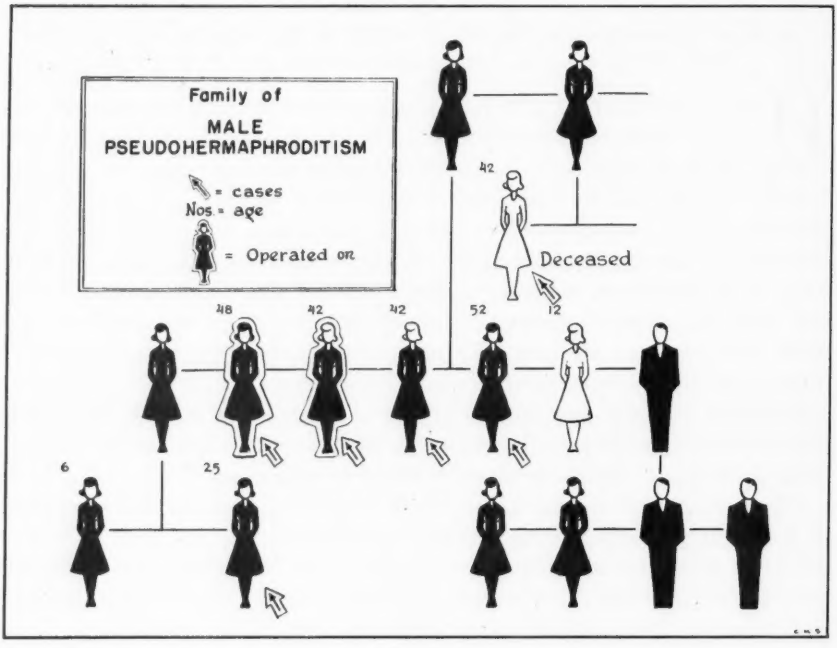

FIGURE 1

Certain theoretical considerations regarding the endocrinologic features of the condition, including hormonal assays, will be presented.

METHODS

The assays of urinary gonadotropic hormone were carried out according to the mouse uterine weight procedure of Klinefelter, Albright and Griswold (1). Immature female mice were used and the gonadotropin to be tested was precipitated from the urine by alcohol. One unit was considered the amount of the hormone which, when injected in five doses over a 3-day

period in a dilution of 0.5 cc. per dose, caused a minimal uterine weight increase. The uterus was considered enlarged if it weighed more than 7 mg.

In the determination of 17-ketosteroids, 24-hour specimens were used. The urine was acidified by the addition of 5 cc. of concentrated sulfuric acid per 100 cc. of urine and immediately hydrolyzed by boiling for ten minutes. The hydrolyzed urine was then extracted in three half-hour periods with redistilled butyl ether, using 200 cc. per liter of urine for each extraction. The combined butyl ether extracts were washed several times with 1.5 N sodium hydroxide solution, and then several times with tap water until the ether layer was clear. Fractionation was made by absorption on magnesium oxide (2). Colorimetric determinations were made by the Holtorff-Koch modification (3) of the Zimmermann reaction (4, 5).

Vaginal smears were obtained according to the method of Papanicolaou and were stained with Shorr's trichrome stain.

CASE REPORTS

Of 5 sisters in the family, 4 had amenorrhea. The fifth sister was apparently normal; however, of 2 daughters, aged 6 and 25, the latter had never menstruated. One maternal cousin, who died at the age of 42, had primary amenorrhea. All of these 5 relatives were of normal female contour, and each had either scant or absent axillary and pubic hair. One brother was married and has had 4 normal children.

Case 1. A 48-year-old, white, married woman, whose presenting complaint was low backache of one year's duration, gave an additional history of primary amenorrhea (Fig. 2). On examination the patient was well developed and well nourished. Her height was 64 inches and her weight, 138 pounds. Her external features were entirely feminine, as were her speech, demeanor and mannerisms. Axillary hair was absent; vulvar hair was extremely scant and fine. The breasts were well developed except for small nipples and areolae. The external genitalia showed labia which were normal in appearance but slightly smaller than average in size (Fig. 4). The clitoris was not enlarged and was well formed. The vagina was shortened to about two thirds average length and ended in a blind pouch. At the end of this pouch, in the position normally occupied by the cervix, a small wrinkling depression was evident; but no cervix or external os could be identified. The urethra was in the normal location. On bimanual examination, a large rounded incarcerated cystic mass was found to occupy the pelvis, but no uterus could be identified. The remainder of the general physical examination showed nothing abnormal. Libido and coital relationships were natural and there was no evidence of homosexual tendencies.

Laboratory data disclosed a normal urine, blood count and sedimentation rate. The concentration of blood sugar was normal. Wassermann and Kahn tests gave negative results. The urinary gonadotropin (FSH) assay showed between 13 and 105 mouse units per twenty-four hours, and 17-ketosteroids measured 5.2 mg. per twenty-four hours

(Table 1, Case 1). Vaginal smears showed a 2 plus to 3 plus estrogen effect (normal, 4 plus).

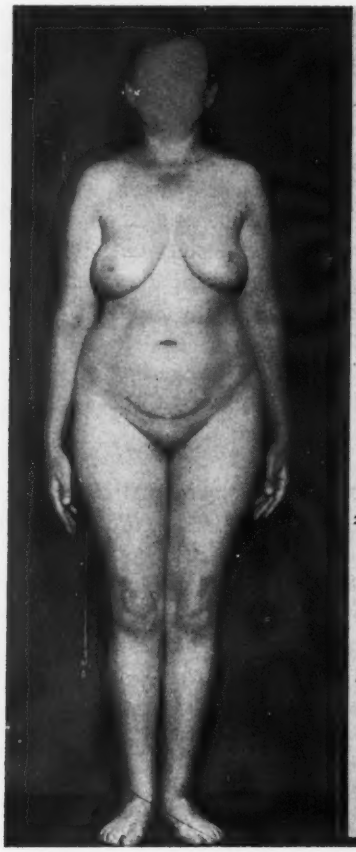

FIGURE 2
(Case 1)

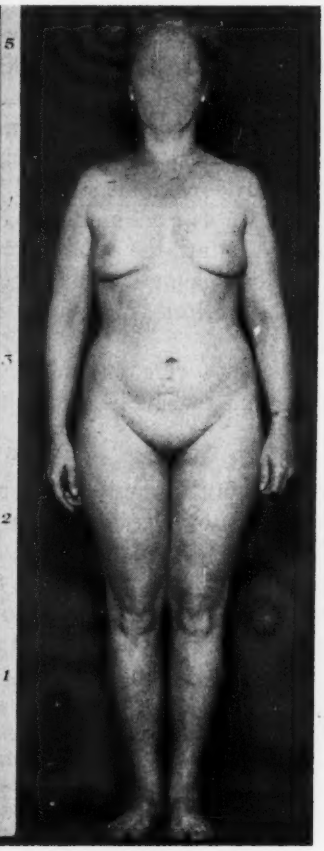

FIGURE 3
(Case 2)

The 2 patients who were operated on. Note similarity of body contour, small nipples, well formed breasts and absence of pubic hair.

Pelvic laparotomy was performed on May 12, 1950, at which time a right gonadal cyst, approximately the size of a small grapefruit, was removed, along with the gonad. Left perigonadal cysts were excised and a wedge biopsy specimen was taken from the left gonad. The pelvic findings are shown in the accompanying illustration (Fig. 5) after removal of the cyst (Fig. 6).

The microscopic sections of the areas containing gonadal tissue showed tubular structures the size and shape of immature testicular tubules. These were formed by cells of uniform character arranged perpendicularly to the long axis of the tubules, with pale-

FIG. 4. (Case 1). Close-up of vulva, showing sparse, fine-textured vulvar hair and normal external genitalia.

TABLE 1

Cases	Urinary gonadotropin, m.u./24 hrs.	Urinary 17-ketosteroids, mg./24 hrs.
Normal	Men & Women 13–105	Men: 7–14 Women: 4–8
Case 1 N.M.	13–105	5.2
Case 2 M.B.	13–105	4.3
Case 3 B.C.	13–105	7.5
Case 4 A.B.	13–105	7.3

staining, ill defined cytoplasm and with nuclei that were oval, slightly irregular, indented, and with a finely granular, evenly distributed chromatin. Several of the tubular structures were formed by cells identical in all respects to Sertoli cells. Although small, ill defined lumina were present in some of these tubular structures, the others were of a solid appearance. There was no evidence of spermatogenesis. Between the somewhat closely approximated tubular structures were many polyhedral cells resembling Leydig cells, of small size with pink-staining cytoplasm and uniform, round nuclei. There was some

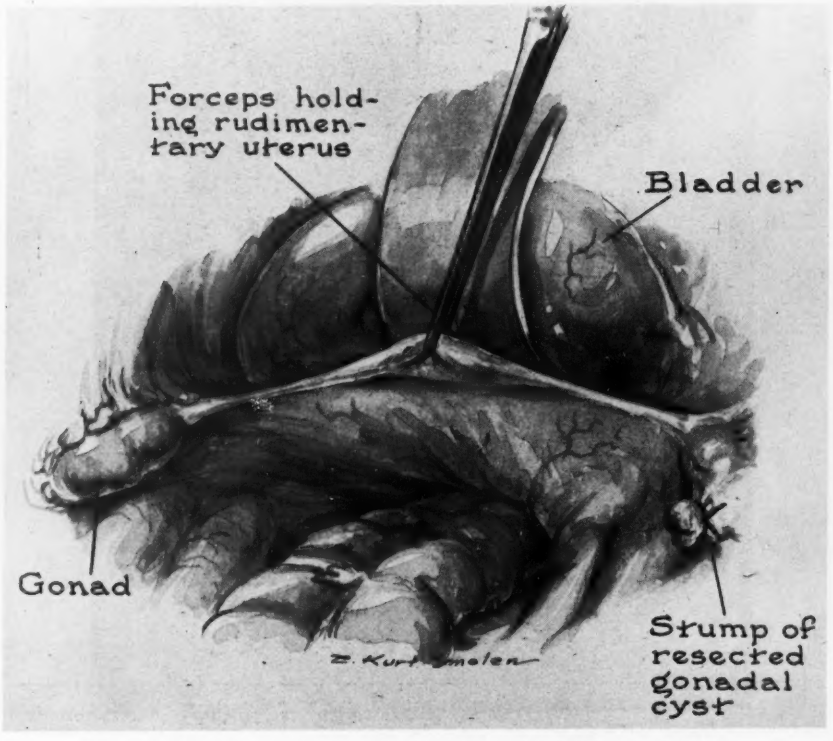

FIG. 5. (Case 1). Artist's reproduction of pelvic viscera at operation, after cyst was removed.

dark-staining collagenous connective tissue similar to ovarian stroma but no functioning ovarian tissue was evident (Fig. 7).

A biopsy specimen of the gonaduct showed a diminutive, rudimentary Fallopian tube with a well formed wall and with villi of the usual character, except for size (Fig. 8).

The large paroophoron cyst was formed by fibrous tissue and lined by a single layer of cuboidal or low columnar dark-staining cells of uniform appearance.

The 2 next youngest sisters (twins) obligingly presented themselves for examination at our request. The findings (*see* Cases 2 and 3) proved identical with those in Case 1. This, incidentally, represents the eleventh

recorded instance in which hermaphroditism of any type has occurred in twins.

Case 2. A 42-year-old divorcée, with primary amenorrhea, was a well developed, well nourished, white woman with no axillary hair and extremely scant, finely textured vulvar hair (Fig. 3). The breasts were fully developed, except for rather small nipples.

FIG. 6. (Case 1). Gross appearance of cyst removed from end of right gonaduct.

The labia were slightly smaller than normal, as was the clitoris. The urethra was in the normal position. The vagina was shortened, with no visible cervix or external os. A small rudiment of the uterus could be felt on bimanual examination.

Laboratory studies revealed a normal urine and blood count. The concentration of blood sugar was normal. Wassermann and Kahn tests gave negative results. A 24-hour urinary gonadotropin assay showed more than 13 and less than 105 mouse units. The 17-ketosteroid assay was 4.3 mg. per twenty-four hours (Table 1, Case 2). The vaginal smear showed a 2 plus estrogen effect.

Subsequently the patient returned with a four-day history of severe pain in the left

lower abdominal quadrant, associated with nausea and vomiting. Examination revealed exquisite tenderness in the left groin with local bulging. With the patient standing, a non-reducible soft tissue mass could be palpated in the inguinal canal. A diagnosis of strangulated hernia, probably containing a testis, was made. An inguinal hernia was also present on the right but its contents were reducible.

Emergency surgery revealed bilateral sliding-type herniae containing structures resembling gonads (Fig. 9). A biopsy specimen was taken from the left gonad before repair of the herniae.

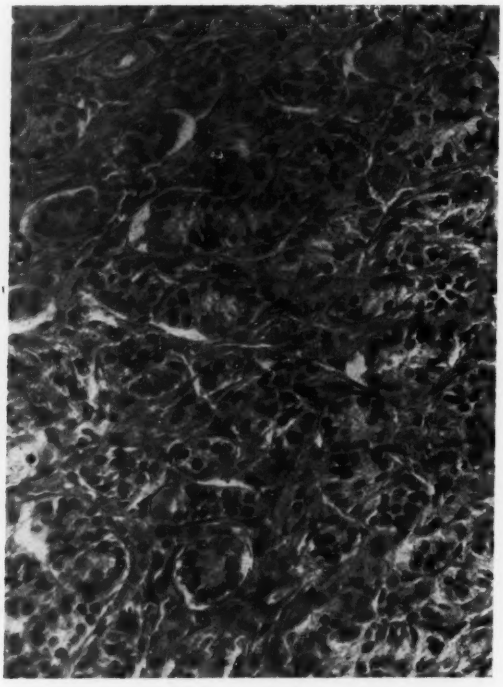

FIG. 7. (Case 1). Microscopic appearance of gonadal tissue (×200).

The microscopic section of the gonadal biopsy specimen revealed findings similar to those described in Case 1.

Case 3. A 42-year old registered nurse with primary amenorrhea had been married for fourteen years. Normal satisfactory sexual relationships and normal libido had been present. In 1932 an appendectomy had been performed, at which time the surgeon stated that the patient had a rudimentary bicornate uterus, and a cyst of a gonad. The general physical and pelvic examinations yielded findings which were identical in all respects to those of her sisters. There were no demonstrable herniae. Routine laboratory studies gave normal results, and the urinary gonadotropin assay showed between 13 and 105 mouse units per twenty-four hours. The 17-ketosteroid assay was 7.5 mg. per twenty-four hours (Table 1, Case 3). The vaginal smear showed a 2 plus estrogen effect.

Case 4. Another sister, who was unmarried, could not be persuaded to have a general physical examination. She had been told that her primary amenorrhea was associated with a short vagina and that no cervix was visible. The uterus was also said to be rudimentary. Axillary and pubic hair, according to the other 3 sisters, was absent in this woman. Assays on 24-hour urine specimens showed between 13 and 105 mouse units of gonadotropin, and 7.3 mg. of 17-ketosteroids (Table 1, Case 4).

Case 5. A maternal cousin, who died at the age of 42, had been of normal feminine development externally and had primary amenorrhea. Pubic and axillary hair had been

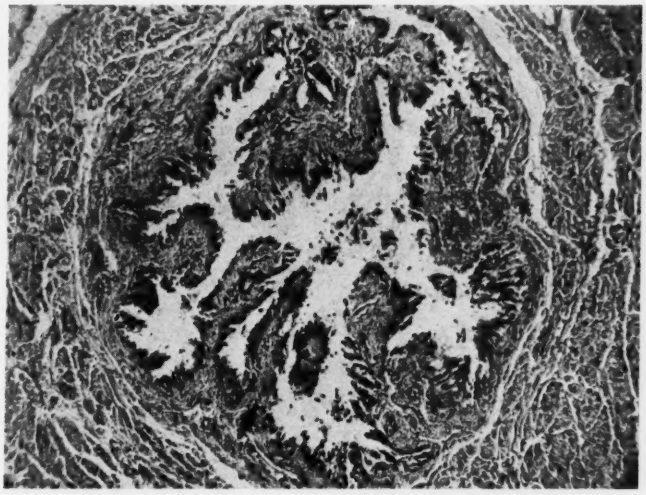

FIG. 8. (Case 1). Microscopic section through right gonaduct.

absent. It had been reported that the vagina was shortened and contained no visible cervix, and that the uterus was vestigial.

Case 6. A 25-year-old niece, the first born child of the normal sister, had primary amenorrhea and absent axillary and pubic hair. She had never been examined by a physician, but it was assumed by all members of the immediate family that her condition was identical with that in the other cases herein reported. Urine could not be obtained for gonadotropin and 17-ketosteroid assays, and physical examination was refused.

DISCUSSION

In 1948 Goldberg and Maxwell (6) reported 1 patient exhibiting complete feminine habitus without signs of masculinity, amenorrhea accounted for by the absence of the cervix and an abnormally developed uterus, and testes in lieu of ovaries in the intra-abdominal position. Axillary and pubic hair were absent. In their review of hundreds of instances of pseudohermaphroditism in the available literature they were able to bring to light only 7 cases (7–11) in which the foregoing criteria were fulfilled. Since their report,

we succeeded in finding reports of 6 similar cases (12, 13) in addition to our 6.

Goldberg and Maxwell assumed originally that the basic lesion was a highly differentiated form of arrhenoblastoma of the type described by Meyer (14) but careful search, both grossly and microscopically, showed no vestige of ovarian tissue. Thus the diagnosis was neither arrhenoblastoma nor ovotestis. The findings at the time of laparotomy and by micro-

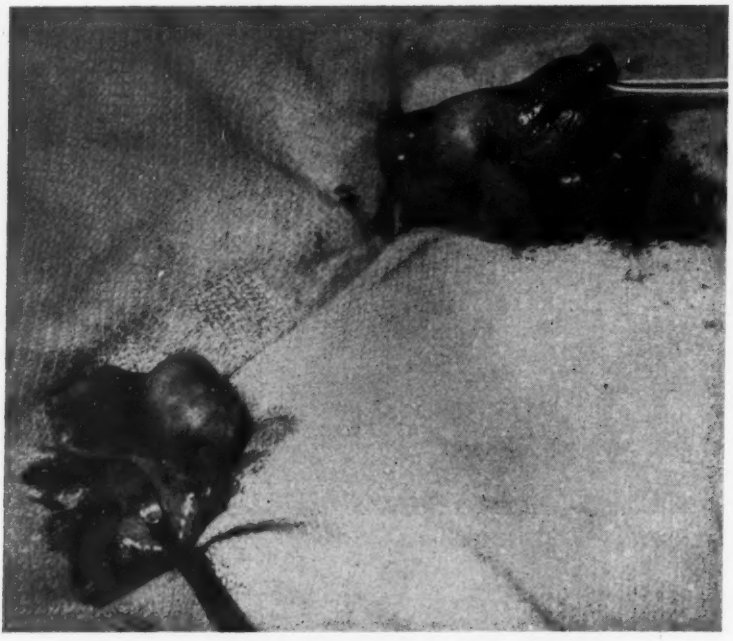

FIG. 9. (Case 2). Photograph of both testes, each contained in its respective inguinal canal. Note infarcted area in left epididymis.

scopic examination showed that the gonads, in the case reported by Goldberg and Maxwell, were represented by immature sterile cryptorchid testes containing adenomatous formations and tubules of small caliber without spermatogenesis. Their conclusion was that their patient was an intersexed individual; the syndrome was classifiable as male pseudohermaphroditism with Müllerian derivatives and complete agenesis of the Wolffian system.

It is apparent that the cases herein described are identical to that of Goldberg and Maxwell. Indeed, familiarity with the clinical features of their reported case was the basis on which the preoperative diagnosis was made possible in our first surgical case. This is emphasized because we feel

that the criteria for the diagnosis of male pseudohermaphroditism of this type can be quite rigidly formulated. The absence or almost complete absence of axillary and pubic hair in the presence of a feminine habitus, amenorrhea and a blind vaginal pouch, assure the presumptive diagnosis of male pseudohermaphroditism with intra-abdominal testes and agenesis of the Wolffian system. It is pertinent, however, that the testes may be present in the inguinal canals, as is illustrated in our second case and as has been so adequately described by others (13, 15, 16). Careful review of the literature in surgical cases shows the constancy with which these clinical signs confirm the findings at the time of pelvic laparotomy. For these reasons we believe that the other members of the family described here belong to this same syndrome, although final proof from surgical intervention is lacking in 4 of the 6 cases. This seems a reasonable assumption and comprises the largest series of male pseudohermaphroditism of this type in one family so far reported.

We feel that the clinical features characteristic of this type of intersexuality need further elaboration. The combination of primary amenorrhea, a blind vaginal pouch, and scant to absent axillary and pubic hair in persons with a thoroughly feminine habitus and no signs of masculinity, demonstrates a remarkable and memorable clinical picture. The adequately described cases in the literature all disclose either a complete absence of axillary and pubic hair or extremely scant, finely textured hair which does not grow to average length. One exception to this has occurred and will be mentioned later. The breasts are ordinarily of normal size; specific reference to small nipples and, at times, small areolae has been made. In our cases small nipples and small areolae were characteristic. The vagina is shortened and the cervix absent, with the fundus either unidentified, or present as a small, abnormally developed vestige, as noted in all of the established cases. The labia are normal in shape, but smaller than average in size. The clitoris is not enlarged; the urethra is in the normal location.

The testicular gonads occupy either an intra-abdominal position (usually in the location of the ovary) or are contained within the inguinal canal. The gross appearance of the gonad frequently resembles that of an ovary, and may explain the failure of recognition of the true nature of the gonad in cases in which biopsies have not been performed. In 6 cases (12, 13, 15, 16), fully authenticated, the gonads were demonstrable in reducible inguinal herniae, as in our second case. The formation of cysts within these gonads seems of relatively common occurrence, since it has been described in 4 of the reviewed cases, and was present in 1 of our 2 surgical cases. It seems to us remarkable that cyst formation should occur in intra-abdominally located testes of this type, since it is not commonly seen in normally placed testes, or in cryptorchid men.

The patients of Bleyer (12) and of Ward-McQuaid and Lennon (13) were operated upon primarily for the repair of inguinal herniae. Biopsy specimens were taken from the gonads found in the hernial sac and the diagnosis of male pseudohermaphroditism was thus incidentally established. This procedure is emphasized because other cases in the literature have been reported for the purpose of demonstrating unusual contents in inguinal herniae, such as "ovaries." In the description of such cases the clinical features are identical to those here described; thus we are convinced that these are unrecognized instances of male pseudohermaphroditism, in reality belonging to this same group. In these patients the gonads were returned to the abdominal cavity, without biopsy.

The literature contains several references to the familial incidence of this condition. It is a feature of the cases described by Ward-McQuaid, by Bleyer, and by our own report. In the instance related by Weisman and Schwarz (11), the statement was made that one younger unexamined sister of the reported patient had never menstruated.

The correct preoperative diagnosis of this condition has been made in only 2 instances in the literature. As previously stated, in our first surgical case our suspicion concerning the diagnosis was aroused by the report of Goldberg and Maxwell. Bleyer correctly predicted the true nature of the condition preoperatively in his second case because of identical findings in a sister in whom the diagnosis had been made at operation previously.

The psyche has always been feminine in these persons. They manifest no evidence of homosexuality or other abnormal sexual inclination. Their libido is usually normal or slightly increased.

Additional clinical features presented by this type of male pseudohermaphroditism bear further comment from an endocrinologic viewpoint. The one striking characteristic demonstrated by the microscopic examination of the gonad is its testicular architecture. No functioning ovarian tissue can be discerned. These gonads contain small calibered tubules without spermatogenesis, with Sertoli cells and Leydig cells. In spite of this, well developed breasts and moderate degrees of vaginal cornification are present and, in the cases herein described, the presence of a normal amount of follicle-stimulating hormone in the urine. These findings, plus the lack of masculinity, indirectly suggest that these testes are producing estrogen. In Wilkins' case, bilateral gonadectomy was followed by the disappearance of "estroids" from the urine, these having been present prior to surgery in amounts estimated at 34 gamma per day by the Kober reaction; simultaneously, a rise in urinary FSH from 96 to 288 m.u. per day occurred. The appearance of hot flashes and atrophy of the breasts following gonadectomy further supports the impression that these gonads are excreting estrogens; such symptoms can be successfully treated by the administration

of estrogen. Furthermore, if the gonads in these persons were functioning in the manner of normal adult testes, one would anticipate 17-ketosteroid levels approximating the range in normal adult men.

It is interesting to speculate upon the source of the estrogen production by the testes in cases of this type. Both the Sertoli cells and the Leydig cells have been implicated as possible sources of estrogen production by the testes. The evidence involving the Sertoli cell as the source of estrogen production is summarized by Hamblen *et al.* (17). Huggins and Moulder (18) have demonstrated the production of estrogen in Sertoli cell tumors in dogs. Teilum (19) has described an estrogen-producing Sertoli cell tumor of the testis in man. Conversely, Nelson (20) has reported studies on 5 individuals similar to those under discussion, in whom the evidence indicates the Leydig cell as the source of estrogen. Histologic and histochemical studies showed well formed Leydig cells in all cases and in 3 cases the Sertoli cells were undifferentiated. Gonadectomy was followed by a decrease in the level of estrogen excretion and was accompanied by "estrogen withdrawal symptoms." Maddock and Nelson (21) have reported further convincing evidence indicating the Leydig cell as a source of estrogen. Normal males were found to excrete increased amounts of estrogen following the administration of chorionic gonadotropin. Testicular biopsy specimens revealed an increase in the number of Leydig cells with no significant changes in the Sertoli cells. Cole *et al.* (22) have directed attention to the fetal horse testis, which is composed predominantly of masses of interstitial cells and shows an unusually high concentration of estrogens.

A practical consideration in the management of these cases is the question of castration. Castration is advocated in order to circumvent neoplasia of cryptorchid testes, to facilitate estrogenization, to avoid possible abnormal steroid metabolism and to benefit the patient psychologically. In many types of male pseudohermaphroditism, particularly those manifesting prominent masculine features, this may be the correct surgical approach. However, we feel that in cases such as ours, in which there is evidence that the primary desired hormone produced is estrogen, there is no advantage in gonadectomy. As in previous cases of castration, troublesome and premature menopausal symptoms may result, requiring prolonged replacement therapy.

A further interesting feature of this type of pseudohermaphroditism is the complete, or almost complete, absence of axillary and pubic hair. This finding is almost invariably present in cases of this type but one well documented case is described by Ackerman, Herweg and Allen (14) in which normal amounts of axillary and pubic hair were present. This is the only exception with respect to this feature, in so far as the authors are aware. In this connection, it is noteworthy that occasionally normally fertile

women have absence of axillary and pubic hair. However, the association of this feature with a blind vagina and absent uterus is strong presumptive evidence to support the diagnosis of the disorder under consideration.

It has been contended, and we think justifiably, that axillary and pubic hair normally are dependent upon the combined activity of the primary gonad and the adrenal cortex. There is much clinical evidence to support this opinion, including the fact that hair appears in these locations following the administration of either estrogen or androgen in the treatment of prepuberal hypogonadism. The loss of axillary hair in women with Addison's disease has been attributed largely to the disappearance, or almost complete disappearance, of 17-ketosteroid production by the adrenal cortex. The appearance of axillary and pubic hair at the time of puberty, concomitant with the appearance of 17-ketosteroids in the urine, has been suggested as further evidence on this subject. Albright (23) has stated, moreover, that all conditions which lead to 17-ketosteroid excretion levels of less than 2 mg. per twenty-four hours (Addison's disease in females, Simmonds' disease, myxedema, and old age) are associated with the disappearance of axillary hair, or at least with its failure to grow back when shaved off.

In the syndrome under discussion, axillary and pubic hair are absent or scant in spite of the presence of a normal amount of 17-ketosteroid in the urine and indirect evidence that the testes are estrogenically active. Goldberg and Maxwell suggested that the absence of axillary and pubic hair in these cases might be explained on the basis of a congenital absence of hair follicles in these locations. However, at the time of the laparotomy in our first case, a biopsy specimen of the skin over the mons pubis showed the presence of diminutive hair follicles; the sweat glands were present in normal numbers and no sebaceous glands were seen. Wilkins *et al.* (15) made an unsuccessful attempt to stimulate axillary and pubic hair growth by replacement therapy. Thus, the lack of growth of hair in these locations must remain unexplained for the present. Although we do not disagree with Albright's theory concerning axillary and pubic hair in most conditions, at least in the syndrome concerned here, this hair is absent despite normal (female) levels of 17-ketosteroid excretion.

SUMMARY

We have reported what we believe to be the largest series of familial male pseudohermaphroditism in the literature. The special clinical features of this syndrome have been presented and the suggestion has been made that, from these observations, one should be able to make an accurate preoperative diagnosis. The salient features include a normal feminine habitus and demeanor, without evidence of masculinity; normally devel-

oped mammary tissue with small nipples; normally developed female external genitalia; absence or almost complete absence of axillary and pubic hair; a vagina less than average in length, ending in a blind pouch without a visible cervix or palpable uterus, although a vestige may be present; and normal (female) urinary excretion levels of 17-ketosteroids and follicle-stimulating hormone. The endocrinologic features of the syndrome have been discussed.

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EFFECTS OF SURGICAL HYPOPHYSECTOMY IN A MAN WITH MALIGNANT MELANOMA*

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MALIGNANT melanoma in man displays a number of features which suggest that its development and growth may be related to or influenced by the endocrine system. Melanomas are almost never malignant before puberty, are not observed in castrated individuals, and their growth and dissemination are markedly accelerated during puberty and during pregnancy (1, 2).

The clinical course of melanoma with metastases is uncompromising in its inexorable progress and cannot be modified by any presently known methods. Attempts to influence the neoplasm by castration, androgenic or estrogenic therapy have been unsuccessful. Pack and Scharnagel (3) performed bilateral adrenalectomy on two occasions without beneficial effects.

Wigby and Metz (4) reported a patient with disseminated melanoma who had striking regression of subcutaneous and visceral metastases of melanoma following roentgen irradiation of the pituitary gland with approximately 4,500 r. No effects were observed, however, in 4 additional patients treated in this manner. Irradiation of the pituitary with up to 3,000 r has shown no effect on melanomas (3). We (5) have treated 1 such patient with a pituitary tissue dose of 8,500 r without modifying the course of the disease. Moreover, doses of irradiation up to 10,000 r to the normal adult pituitary gland have failed to produce any clinical, biochemical or histologic alterations in 3 patients.

Consideration of the available data indicated, therefore, that further studies on the possible relationship of endocrine influences upon the growth of melanoma in man would be facilitated by the surgical extirpation of the hypophysis. Two patients with advanced neoplastic disease underwent

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craniotomy for this purpose, but the presence of meningeal metastases in one patient and the poor general condition in the other prevented successful completion of the operation. The third patient was a 32-year-old man in good general physical condition despite the presence of massive pulmonary metastases from melanoma of an undetermined primary site. He survived for nine weeks following a successful removal of the hypophysis. No effects on the growth of the neoplasm were observed clinically, although at autopsy some unusual degenerative changes were present in the hepatic and splenic metastases.

The present case report is stimulated primarily by its endocrinologic interest, since the patient was extensively studied before and after the procedure from the standpoint of the development of hypopituitary manifestations. Sheehan and Summers (6) refer to 2 previous instances of deliberate operative removal of the apparently normal pituitary in human beings. The case of Chabanier *et al.* (7), of a hypophysectomy in a 24-year-old male with severe diabetes, does not include an autopsy examination. The case of Elden (8), of a hypophysectomy in a 15-year-old female with epilepsy, displayed the classic manifestations of hypopituitarism, although an autopsy six years later revealed that about 10 per cent of the gland was retained.

CASE REPORT

Clinical résumé. *R.L.C.*, a 32-year-old single, white art student, was admitted to the University of California Hospital on December 12, 1950. His chief complaints were a mass on the left side of the neck and vague pains in the right side of the chest, of three weeks' duration.

The patient was in good health until December 1949, when he had an episode of severe colic in the left flank followed by hematuria. He passed a stone in his urine. An x-ray film was stated by the patient to have demonstrated a "cyst at the lower pole of the right kidney and stones in the left kidney." He had no further recurrence of the genito-urinary complaints.

In March 1950 and again in June 1950 he had two sudden episodes of cough and minimal hemoptysis, which persisted for approximately four days. These attacks were associated with vague pains in the chest, particularly anteriorly on the right side. Three weeks before entry there was recurrence of the chest pain on the right side, without cough or sputum, and he noted that a nontender mass had appeared above his left clavicle. This mass had increased in size, was not associated with pain, and was movable. The patient had not lost weight, his appetite remained normal, and he had no fever, night sweats or generalized malaise.

The past history was entirely noncontributory, except for right orchitis due to mumps as an adolescent. X-ray examination in 1945 showed the chest to be normal.

Physical examination showed a well developed, well nourished male. His weight was 70.6 Kg. and his height, 177 cm. Temperature, pulse and respirations were normal. Blood pressure varied between 108/70 and 110/80. There were two hard, nontender, freely movable lymph nodes, about 2 cm. in diameter, in the left supraclavicular fossa. There were small flat pigmented nevi on the left pectorolateral thorax and the deltoid area of the arm. Otherwise, the results of examination were entirely negative.

Laboratory studies of the urine and blood revealed no abnormalities (Table 1). X-ray films of the chest showed circular, clearly defined shadows, 1 to 5 cm. in diameter, in the right lung, as well as two smaller shadows on the left. Intravenous and retrograde pyelograms demonstrated a calculus in the lower pole of the left kidney. Gastro-intestinal series and barium enema failed to reveal abnormalities.

A lymph node was removed from the left supraclavicular area. The mass was 2 cm. in diameter, circumscribed, and black in color. The gross diagnosis of melanoma was substantiated by histologic examination. The two pigmented skin areas were removed also, but histologically showed only actively proliferating junctional nevi.

The clinical diagnosis was, therefore, malignant melanoma, primary site undetermined, with metastases to supraclavicular lymph nodes and the lungs.

After full discussion with the patient, it was decided to perform a hypophysectomy on an experimental basis in the hope of retarding the growth of the neoplasm. It was the opinion of the Consultative Tumor Board that the prognosis for the patient was less than six months.

Surgical procedure. On January 25, 1951, a hypophysectomy was performed. Through a right frontal craniotomy the pituitary fossa was approached between the right optic nerve and the internal carotid artery. The pituitary stalk was clipped and divided, and the pituitary gland freed after incision of the diaphragm of the sella. The pituitary gland was removed *in toto*.

During the following twenty-four hours the patient's condition was satisfactory. At ten hours postoperatively the peak temperature of 39.5°C went down to 38.6, and he began to respond. Soon thereafter, however, he became deeply comatose, both pupils became fixed to light, and the right pupil was larger than the left. The extensor tone was increased in all four limbs, with opisthotonic posture and ankle clonus on the right.

The patient was returned to the operating room, and the wound reopened. A large organized subgaleal and extradural collection of blood was found and removed. The brain was edematous, and the right frontal lobe was partially removed in order to visualize the sella turcica. No hemorrhage or tension were observed, however.

The patient was treated with large doses of cortisone, desoxycorticosterone acetate, testosterone, and hykinone as well as transfusions of whole blood. There was progressive improvement of his status, and eight days postoperatively, he was responding to commands. The main sequela of the operation was a partial right third cranial nerve palsy. The cerebrospinal fluid pressure and hydrodynamics were normal.

Course after hypophysectomy. The course of the patient is indicated in Figure 1 and in Table 1. During the first two weeks after the hypophysectomy, he received exogenous steroids, which included 1.34 Gm. of cortisone administered during six days, eleven administrations of desoxycorticosterone acetate for a total dose of 360 mg., and six injections of testosterone propionate for a total dose of 500 mg. He also received two injections of ACTH for a total dose of 325 mg.

Cortisone and testosterone were discontinued seven days after operation, and no desoxycorticosterone was given after thirteen days. The changes observed during the fourth and fifth weeks, therefore, are of primary interest.

Clinically, the patient displayed a progressive syndrome of hypopituitarism, with apathy, slow responses, somnolence and decreased peripheral reflexes. The basal metabolic rate dropped from −3 to −33 per cent. He remained in bed unless he was urged and helped to get up and sit in a wheelchair; only occasionally did he read his mail. He did not complain of coldness, arthralgia or loss of libido, but satisfactory responses to these questions were not obtainable. It is not known to what extent the postoperative

TABLE 1. DATA ON THE BIOCHEMICAL CHANGES

	Normal range	Preoperative	Week after hypophysectomy									
			1	2	3	4	5	6	7	8	9	Terminal day
			Steroids		No therapy			ACTH			Adrenal extract	
Weight (Kg.)	—	65.6–67.8	—	62.7	—	61–62.2	63–64	—	63.5–58.3	59.3	60.4	—
BMR	−15 to +15	−3	—	—	—	−23	−33	−26	−23	−21	−26	—
Serum iodine (γ/100 cc.)	4–8	6.7–7.0	—	5.0	3.6	3.5–4.2	3.6	4.2	5.0	3.5	3.2	2.8
17-Ketosteroids (mg./24 hrs.)	10–25	7.4–19.8	—	21.8	—	1.9–3.4	—	5.4	1.3	1.4	—	—
Urinary gonadotropins (mouse units/24 hrs.)	10–40	5	—	5	—	5	—	5	5	—	5	5
Blood glucose (mg./100 cc.)	60–80	75–79	73	—	59–69	—	45	59–62	50	52	49	64
Cholesterol (mg./100 cc.)												
Free	total	28–32	—	40	44	40–41	34–40	40–43	42	35	25	23–20
Total	107–320	121	—	118	158	127–151	114–128	117–135	130	85	77	61–32
Serum												
Na (mEq./L)	130–140	137	136–147	144	138–141	135	125	128	131	—	139	137
K (mEq./L)	3.5–5	4.8	4.2–5.0	4.5	5.0	5.1	5.3	—	5.7	6.2	5.6	9.4
Cl (mEq./L)	99–106	105	89–114	110	107	109	100	97–107	110	100	100–114	114
P (mg./100 cc.)	3–4	2.8	—	2.9	2.3–2.8	2.2	2.1–3.0	2.3	3.1	2.7	3.1	6.4
Ca (mg./100 cc.)	9.5–11	—	—	8.9	9.4–9.7	9.2	8.9	8.6	—	8.8	10-2	8.0
Protein (Gm./100 cc.)	5–7.5	6.4	—	5.6	6.1	6.2	6.2	5.7	6.4	6.2	5.4–5.5	4.3
Albumin	3–4.5	3.9	—	2.9	3.5	3.1	3.1	2.8	3.4	3.2	3.0–2.8	2.1
Globulin	1.5–3.4	2.5	—	2.7	2.6	3.1	3.1	2.8	3.0	3.0	2.4–2.7	2.2
Uric acid (mg./100 cc.)	2–4	4.2	—	4.0	4.4	—	3.2–4.2	3.7	3.4	4.7	4.2–6.2	7.7
NPN (mg./100 cc.)	15–30	23	—	24	22	26	28	22	26	29	35–41	61
Alkaline phosphatase (Bodansky units)	1–5	2.0	—	2.2	2.4	2.4	2.8–3.8	3.5	3.3	2.6	2.0	2.4
Thymol turbidity (units)	0–5	8.2	—	6.0	8.5	—	8.4	—	6.2	7.8	7.6	3.5

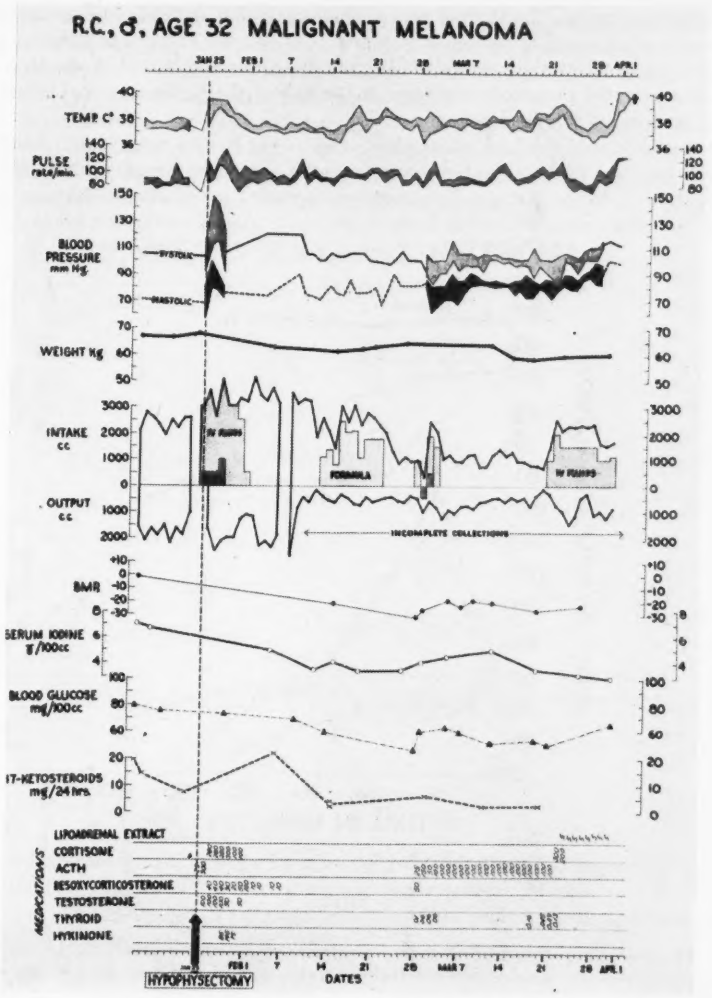

FIG. 1. General data on the clinical course of the patient, including treatment, before and after surgical hypophysectomy. For exact values of chemical determinations, see Table 1.

cerebral edema and the partial right frontal lobectomy contributed to this mental state, but in retrospect the role of cerebral metastases must be strongly considered.

The skin remained of good turgor, although it became progressively drier. No unusual fat deposits developed. The complexion was somewhat sallow and pale, with a suggestion of puffiness around the eyes. The axillary and pubic hair remained normal, as did the axillary secretions; the patient continued to grow a beard at approximately the same

rate as before surgery. The thyroid was normally palpable and did not decrease in size. The prostate remained of the same size and consistency. There was minimal loss of weight, consistent with the progress of disseminated malignant tumor. X-ray films of the chest demonstrated progressive increase in the size of the pulmonary metastases. The cardiac shadow did not change in size.

Determinations of the fluid intake and output of the patient were in part invalidated by occasional loss of urine collections, but suggested a decreased urinary output following the cessation of steroid therapy. The decrease, however, was related to decreased intake and no real effect can be stated to have occurred. The urine during the whole course of

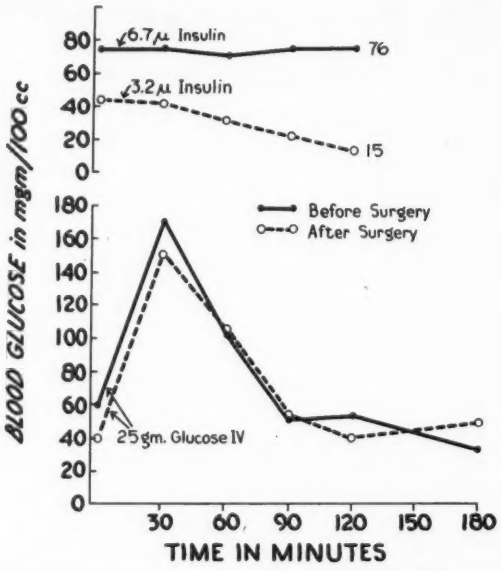

FIG. 2. Glucose tolerance following 25 Gm. of glucose intravenously, before and twenty-one days after hypophysectomy. Insulin tolerance following 6.7 and 3.2 units of insulin intramuscularly, before and thirty-five days after hypophysectomy.

study, including the pre-hypophysectomy period, was of low specific gravity, ranging between 1.009 and 1.012. Except for the occasional presence of a trace of albumin and some pus cells, the urine remained normal. One specimen, during the fourth week, gave a positive result for melanin by the ferric chloride reaction, and then gave negative results until another positive reaction was obtained on the urine collected on the terminal day of illness.

Biochemical observations were in conformity with the clinical evidence of a hypopituitary state. As given in Table 1 and Figure 1, the serum protein-bound iodine level dropped from 6.7 to 3.6 micrograms per 100 cc. by the third week, the fasting blood glucose level dropped from 75 to 45 mg. per 100 cc. by the fifth week, and the urinary ketosteroids (total neutral steroid fraction), after a rise during the second week due to the injections of testosterone, dropped to approximately 3 mg. per twenty-four hours

during the fourth week. There was a decrease in the concentrations of serum sodium and chloride during the fifth week. The serum phosphorus and calcium remained at borderline low levels. Blood cholesterol remained at the same level for seven weeks of observation. Blood proteins were not significantly affected.

A glucose tolerance test was performed before and three weeks after hypophysectomy, using an intravenous dose of 25 mg. of glucose. Figure 2 shows that there was no change in the curves. An insulin sensitivity study showed that before the removal of the pituitary, the intramuscular injection of 6.7 units of insulin resulted in no change in the

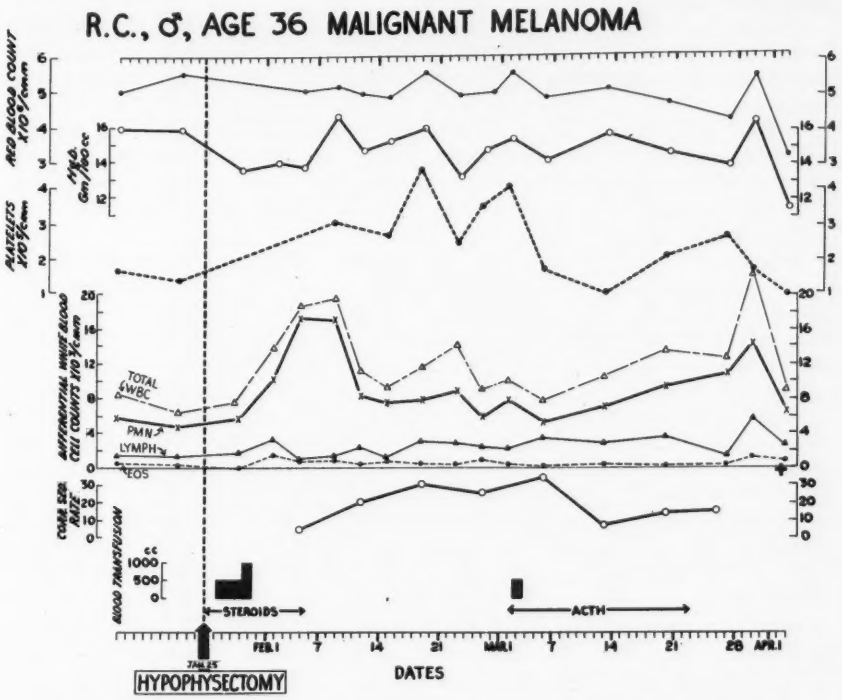

FIG. 3. Hematologic data on the patient, before and after hypophysectomy.

blood glucose; after the hypophysectomy, injection of 3.2 units of insulin resulted in a drop of blood glucose from 45 mg. to 15 mg. in two hours.

The effect of ACTH upon the circulating eosinophils was studied at the completion of the fifth week after operation. Following the administration of 25 mg. of ACTH, the eosinophil count increased from 78 to 188 per cu. mm.; however, there was a reduction of eosinophils from 188 to 61 per cu. mm. following the injection of 50 mg. of ACTH.

Figure 3 presents data on the hematologic findings during the nine weeks of study. No significant alterations were observed in the red blood count, hemoglobin or sedimentation rate. There was an increase in the number of lymphocytes in the blood, as compared with pre-hypophysectomy values. The two rises in granulocytes were attributable to the postoperative effects and to the terminal bronchopneumonia.

The clinical condition of the patient six weeks following hypophysectomy and four weeks after the last administration of steroid hormones suddenly became worse. He responded poorly, the muscle tone and reflexes became increasingly weaker; the pulse was weak and the blood pressure fell. It is considered that this period might have been related to further growth of the cerebral metastases.

Small doses of desiccated thyroid and adrenocorticotropic hormone (up to 100 mg. per day in four divided doses) were cautiously administered. The alarmingly low blood pressures became stabilized at a higher level, his responses improved, and the serum sodium and chloride returned to more normal levels.

The progress of the patient was slowly but steadily downhill during the next three weeks. X-ray films of the chest showed progressive growth of the metastatic lesions. The weight decreased from 64 Kg. to 59 Kg. between the fifth and eighth weeks postoperatively.

During the eighth and ninth weeks he was given lipoadrenal extract (5 mg. per day) and daily intravenous infusions of glucose, without clinical improvement. On the sixty-fourth day following hypophysectomy, some respiratory distress developed, and he died three days later. Death was attributed to disseminated melanoma metastatic to the lungs, and terminal bronchopneumonia.

Autopsy. Examination of the skin of the body, including the fingernails and oral cavity, showed only infrequent, small pigmented nevi, none of which was remarkable in size or color. The hair of the head and that of the left axilla and right groin, which had been shaved off, had regrown and the face had a good beard growth. The breasts were those of a normal male.

The right lung weighed 1,020 Gm. and the lower and middle lobes were massively replaced by spherical tumor nodes measuring 5 to 6 cm. in diameter. The left lung weighed 420 Gm. and the lower lobe contained several 3-cm. black tumor masses. The pulmonary tissue surrounding the black tumor masses was congested and edematous. The heart weighed 270 Gm. and was normal.

Masses of tumor cells replaced and invaded the pulmonary parenchyma, which showed a focal peribronchiolar polymorphonuclear exudate and occasional bacterial colonies. The tumor in the central portion showed considerable fibrosis and the tumor cells appeared somewhat small and shrunken. At the periphery the tumor was more cellular. The cells varied greatly in size and shape, with basophilic cytoplasm and irregular polyhedral or spindle-shaped boundaries. In most regions the cells contained many melanin granules, but the amount of pigment varied greatly from area to area.

The liver weighed 1,540 Gm., and contained two black tumor nodules measuring 1 to 2 cm. in diameter in the left lobe. The liver cells were rather small but the architecture was well preserved. Sections through both brown nodules showed deeply brown melanin pigment granules embedded in a dense collagenous scar tissue, with an intact fibrous capsule. No tumor cells could be identified. Stains for iron gave negative results.

The spleen weighed 320 Gm. and the cut surface showed several large black tumor masses, measuring up to 5 cm. in diameter. Histologically, the spleen showed congestion but was otherwise normal. Sections through the tumor showed the cells to be remarkably small and shrunken in appearance, and the center of the tumor mass completely hyalinized and necrotic. Even at the periphery of the neoplasm there was considerable interstitial fibrous tissue and the tumor cells appeared degenerated, with hydropic nuclei.

The gastro-intestinal tract was normal with the exception of a single semipedunculated 3-cm. tumor nodule within the mucosa of the jejunum. The mucosa over this mass

was grossly slightly ulcerated. Histologically, the mucosa of the small intestine was atrophic in appearance and contained a decreased number of mitoses at the base of the glands. The semipedunculated melanotic mass noted grossly in the jejunum was composed of tumor cells.

The right kidney weighed 120 Gm. and the left, 140 Gm. They were pale, the capsules stripped with ease, and were otherwise normal, as were the ureters and bladder.

The retroperitoneal lymph nodes were increased in size, but were soft and of normal

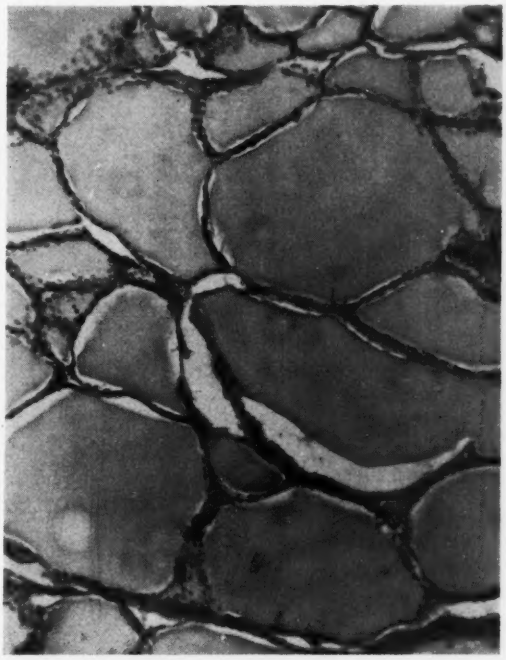

FIG. 4. Thyroid. There is abundant colloid, but the epithelial cells are flattened. (H. and E. ×100.)

color. A single black tumor mass, apparently replacing a lymph node, was found in the retroperitoneal tissue behind the left kidney, and similar lymph nodes were noted in the mesocolon. The lymph nodes uniformly showed a striking lymphocytic hyperplasia with increased size and number of germinal centers.

The bone marrow was cellular and well preserved, with an increased number of eosinophils. A microscopic clump of melanotic tumor cells was observed in one area of bone marrow.

Endocrine system. The thyroid gland was large and pale, weighing 38 Gm. and measuring 7×5×2 cm. The parathyroids were normal. Histologically, the thyroid follicles were strikingly distended with deep-staining collagen, and were lined by scanty, extremely flattened epithelium (Fig. 4).

The pancreas weighed 100 Gm. and appeared normal. Histologically the pancreas was normal, with well preserved islet tissue.

The right adrenal weighed 5.5 Gm., measured 4×3×0.5 cm. and was uniformly reduced in all its dimensions, with a thin cortex and a sparse amount of lipoid material. The left adrenal was encompassed and destroyed by a black tumor mass weighing 180 Gm. A thin portion of intact cortex was found within the tumor. Histologically, the right adrenal showed reduction of all three layers, particularly the fasciculata and reticularis layers. The latter was composed of a narrow band of cortical cells with very

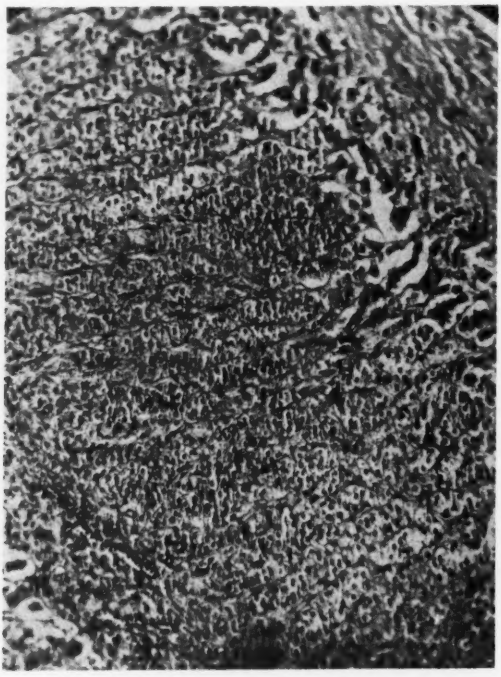

FIG. 5. Adrenal cortex, showing reduction in size, small cellular size and scanty cytoplasmic vacuolization. (H. and E. ×100.)

slight vacuolization of the cytoplasm and pyknotic nuclei (Fig. 5). The medulla was well preserved and cellular.

The left testis weighed 12 Gm. and was grossly well preserved; the right testis was fibrotic (history of mumps orchitis). The left testis showed a striking hypocellularity of the germinal epithelium; spermatogenesis was practically absent (Fig. 6).

The prostate and seminal vesicles were grossly of normal size and appearance. Histologically, however, the epithelium of the seminal vesicles was thin and the villi showed striking submucosal hyalinization and fibrosis (Fig. 7). The prostatic glands were diminished in size and frequency, and were embedded in an abundant, slightly edematous fibromuscular stroma. The cells lining the glands were flattened.

Head. The brain weighed 1,470 Gm. The region of the optic chiasm was normal and

the base of the hypothalamus was normal except for the absence of the pituitary stalk and of the pituitary gland.

Black tumor masses, 1 to 3 cm. in diameter, were noted in the right superior frontal gyrus, right supramarginal gyrus, left cingulate gyrus, right cerebellar cortex and the inferior vermis, the latter partially occluding the fourth ventricle. Histologically, the tumor was well preserved and was actively proliferating.

The entire sella turcica was removed as one segment. The entire fibrous contents of the fossa and the decalcified bone of the sella were fixed and removed for histologic

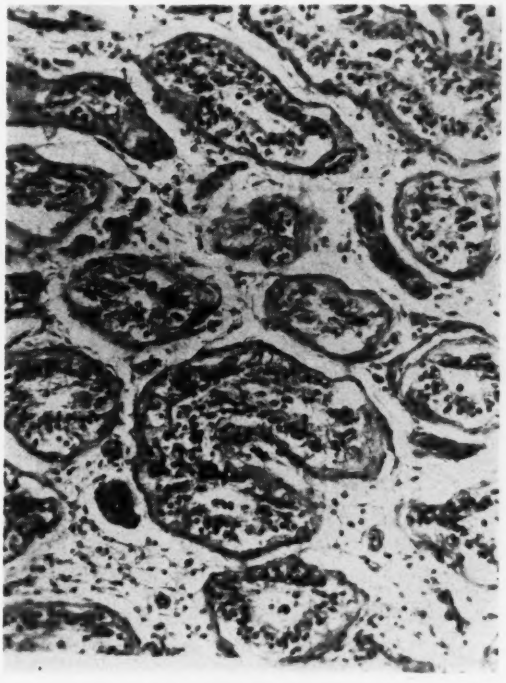

FIG. 6. Testis, showing marked regressive changes in the germinal epithelium. Mature sperm are absent. (H. and E. ×100.)

sections. The orbital contents and the nasopharynx were explored for tumor, but no masses or pigmented areas were identified.

Sections through the fibrous contents of the pituitary fossa showed one focal area consisting of a thin cell layer lying in the planes of the dural lining of the sella. The group of cells were perhaps two dozen in number. Mallory-Azan stains readily identified the cells as pituitary in origin (Fig. 8). The cells observed were largely chromophobes, with rare acidophils and basophils, the former having a degranulated appearance. The posterior lobe of the pituitary was completely absent.

The anatomic diagnosis was 1) melanotic melanoma, primary site undetermined, with widespread metastases to lymph nodes, lungs, liver, spleen, left adrenal, cerebral cortex, bone marrow and the jejunum, with massive necrosis and fibrosis of the tumor in the

liver and spleen, 2) surgical hypophysectomy, with a microscopic cell rest of pituitary cells in the dural lining of the pituitary fossa, 3) epithelial atrophy of the right adrenal, left testis and thyroid, 4) partial resection of the right frontal lobe of the brain, 5) fibrosis of the right testis, and 6) bilateral bronchopneumonia.

DISCUSSION

Sheehan and Summers (6) describe the clinical and pathologic features

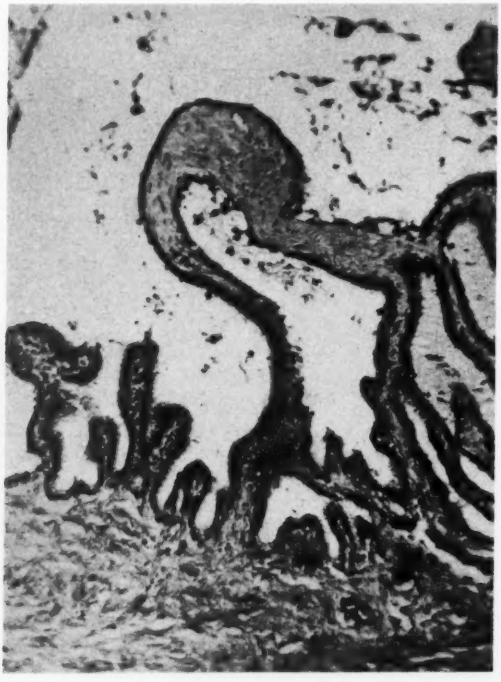

FIG. 7. Seminal vesicle, showing regressive changes of the epithelium with fibrous thickening of the stroma. (H. and E. ×100.)

of hypopituitarism so fully and critically that no further review of the topic is indicated here. Following sudden destruction of the gland by ischemic necrosis, it is stated that "for the first few weeks there is little evidence suggesting an endocrine deficiency." In the present patient, in whom more than 99 per cent of the entire hypophysis was surgically removed, a definite drop in 17-ketosteroid excretion, serum iodine, blood glucose and basal metabolic rate occurred within one month after surgery and within two weeks after the last administration of exogenous steroids.

The patient continued to demonstrate the signs and symptoms of pan-

hypopituitarism during the sixty-seven days of observation. It is unknown, of course, to what extent the clinical course was modified by the presence of the disseminated melanoma. The cerebral metastases must have been at least in part involved in the apathy and lack of responsiveness of the patient.

The absence of some findings associated with panhypopituitarism in the patient is attributable to the relatively short period of observation. Thus,

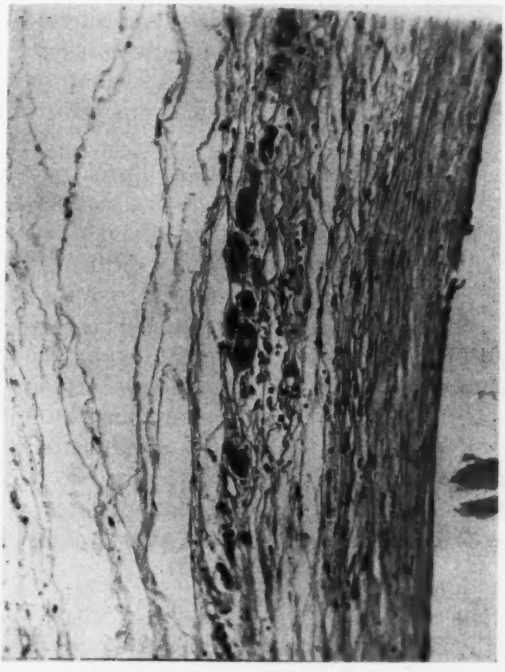

FIG. 8. Residual pituitary tissue found at autopsy. A thin strand of glandular cells remains in the fibrous tissue overlying the sphenoid bone. (H. and E. ×100.)

loss of axillary and pubic hair, diminution in sweating and normal moisture of the axillae, and the clinical appearance of fully developed pituitary myxedema were not to be anticipated for a considerably longer period than sixty-seven days after the removal of the hypophysis (6, 8).

In regard to the biochemical alterations, as summarized in Table 1, it is worthy of note that the potassium and calcium remained at the borderline low normal values (9). The decreases in the levels of serum sodium and chloride during the fifth week were apparently corrected by the administrations of adrenocorticotropic hormone, although this treatment caused

but a transitory slight rise in the urinary ketosteroid excretion (9). There was also a rise in the serum protein-bound iodine level; the presence of thyrotropic hormone in the adrenocorticotropic preparation can be postulated to explain this effect. The marked drop in the cholesterol content of the blood during the last two weeks may be attributed to the low caloric intake of the patient (10).

The patient demonstrated the relative and absolute increase in the number of lymphocytes in the peripheral blood, as seen in panhypopituitarism (11). At autopsy, there was definite gross and microscopic generalized hyperplasia of lymph nodes (12). The endocrine organs were not grossly atrophic, but histologically demonstrated epithelial atrophy (6). The large size of the thyroid was presumably due to a preoperative colloid storage goiter.

In all, the case shows that the clinical and histologic manifestations of panhypopituitarism in man develop within one and two months, respectively, following the removal of the pituitary gland.

The possible effects of the hypophysectomy on the growth of the metastatic melanoma cannot be considered seriously from the study of one case. It can be said that the metastatic lesions in the lungs grew at the same rate during the month of observation previous to hypophysectomy as during the period following the operation. The massive necrosis and fibrosis of tumor nodules in the liver and spleen were unusual, and the possibility that these changes were associated with the experimental procedure should be considered tentatively until additional cases become available. The present conclusion, on the basis of a single patient observed for nine weeks, is that hypophysectomy did not retard the growth of the tumor.

SUMMARY

Surgical hypophysectomy was performed on a 32-year-old man with metastatic melanoma. The patient survived for nine weeks. Within a month after surgery and two weeks after postoperative steroid therapy, panhypopituitarism developed, as manifested by a drop in the basal metabolic rate, protein-bound serum iodine concentration, blood glucose, and urinary 17-ketosteroid excretion. Autopsy revealed epithelial atrophy of the testis, adrenals and thyroid, and generalized hyperplasia of lymph nodes. A microscopic cell nest of adenohypophyseal cells was found in the pituitary fossa; the posterior pituitary had been completely removed.

Hypophysectomy in this patient had no definite effect on the progressive growth of the melanoma, although unusual degenerative changes were found in metastases to the liver and spleen.

Acknowledgment

We are indebted to Drs. Roberto F. Escamilla, Gilbert S. Gordan and W. Eugene Stern for consultative and clinical assistance in the management of the patient.

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ACTH AND CORTISONE *VS.* SALICYLATES

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BEFORE the cortisone-ACTH era, salicylate therapy was one of the most effective means of managing rheumatic fever and arthritis. Because of this observation, the question has been raised as to whether or not salicylates operate through the pituitary-adrenal mechanism.

The accompanying table is an attempt to tabulate the biologic similarities between cortisone and ACTH on the one hand, and salicylates on the other. It is obviously impossible to incorporate in a table the various shades of meaning and interpretation, and the limitations which characterize each report cited. However, the summary should provide a stepping-off place for those interested in the field and, further, might serve to stimulate others to delve into this problem who have not been aware of it. It appears that the many similarities between the two forms of therapy might be more than coincidental and the evidence suggests that further studies bearing on this relationship are desirable.

TABLE 1

Criterion	Biologic effects of ACTH and Cortisone *vs.* Salicylates	
	Similarities	Differences
Adrenal ascorbic acid and cholesterol	ACTH—Causes fall in adrenal ascorbic acid. SALICYLATES (Blanchard, *et al.*; *Bull. Johns Hopkins Hosp.* **86**: 83, 1950.) Salicylates cause significant fall. Prevented by hypophysectomy. Other compounds have no effect indicating specificity. (Hetzel and Hine: *Lancet* **2**: 94, 1951.) Salicylates cause significant fall. Prevented by hypophysectomy. Other compounds less effective. (Robinson: *Brit. M. J.* **1**: 300, 1951.) Aspirin (30 mg. per 100 grams body weight) in rats causes depletion in adrenal cholesterol. (VanCauwenberge: *Lancet* **2**: 374, 1951.) Hypophysectomy abolishes fall in adrenal ascorbic acid and cholesterol caused by salicylates.	
Liver glycogen		CORTISONE—Causes increase in liver glycogen. SALICYLATES (Lutwak-Mann: *Biochem. J.* **36**: 706, 1942.) A dose of 1 mg. of salicylate per rat causes fall in liver glycogen from 3–4% to 0.2–0.4% (Ingle: *Proc. Soc. Exper. Biol. & Med.* **75**: 673, 1950.) Aspirin reduces glycosuria in diabetic rats.

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TABLE 1—*Continued*

Criterion	Biologic effects of ACTH and Cortisone *vs.* Salicylates	
	Similarities	Differences
Eosinophils	ACTH and CORTISONE—Cause fall in circulating eosinophils. SALICYLATES (Roskam and VanCauwenberge: *Lancet* **2:** 375, 1951.) A dose of 4 to 6 Gm. of salicylate in man causes 50% decrease in eosinophils at 6 hrs. No significant change at 4 hrs.	SALICYLATES (Meade and Smith: *Lancet* **1:** 773, 1951.) Salicylates given in a dose sufficient to produce a plasma concentration of 38 mg. per 100 cc. do not significantly depress the eosinophils at 4 hrs.
17-*Ketosteroid excretion*	ACTH and CORTISONE—Cause increase in urinary 17-ketosteroids. SALICYLATES (Bertolani, *et al.*; *Lancet* **1:** 54, 1951.) Salicylates cause increased output of urinary 17-ketosteroids in guinea pigs.	
Antirheumatic effect	Before the advent of ACTH and cortisone, salicylates were one of the most effective agents in the treatment of rheumatic fever and rheumatoid arthritis.	
Anti-inflammatory effect	ACTH and CORTISONE (Selye: *Brit. M. J.* **2:** 1129, 1949.) ACTH and cortisone inhibit arthritis induced by intra-articular injection of formalin. SALICYLATES (LaBelle and Tornaben: *Science* **114:** 187, 1951.) Aspirin reduces joint swelling produced by intra-articular injections of silver nitrate.	
Antipyretic effect	ACTH and CORTISONE It is now well established that ACTH and cortisone reduce the body temperature in the connective tissue diseases and also in infectious diseases, such as pneumonia. (Recant, *et al.*; *Proc. Soc. Exper. Biol. & Med.* **75:** 264, 1950.) Febrile response of rabbits to vaccines is 1.2° to 1.5° F. less in a cortisone-treated group. SALICYLATES—Antipyretic effect well established. (Warren and Werner: *J. Am. Pharm. A.* **35:** 257, 1946.) Increased temperature due to pyrogens is reduced as follows: By aminopyrine..... To 3° below normal By acetanilid..... To 1° below normal By aspirin..... To normal	
Analgesia	ACTH and CORTISONE The ability of ACTH and cortisone to relieve joint pain is well established. (Grokoest, *et al.*: *J. Clin. Investigation* **30:** 644, 1951.) ACTH and cortisone have no analgesic effect. (Lee and Pfeiffer: *Proc. Soc. Exper. Biol. & Med.* **77:** 753, 1951.) Cortisone does not elevate pain threshold in man. SALICYLATES Salicylates have long been classified as analgesics. However, they have certain limitations. For example, Goodman and Gilman state that "the types of pain amenable to relief by salicylates are headache, myalgia, arthralgia and other pain arising from integumental structure rather than from viscera. The significance of this is poorly understood." (Sonnenscheim and Ivy: *J. Pharmacol. & Exper. Therap.* **97:** 308, 1949.) Aspirin does not elevate tooth pain threshold. (Berrien, *et al.*: *J. Pharmacol. & Exper. Therap.* **100:** 67, 1950.) Aspirin has no analgesic effect. (Lee and Pfeiffer: *Proc. Soc. Exper. Biol. & Med.* **77:** 753, 1951.) Aspirin does not elevate pain threshold in man.	SALICYLATES (Bonnycastle, *et al.*: *Acta pharmacol. et toxicol.* **6:** 354, 1950.) Aspirin has analgesic effect.

TABLE 1—*Continued*

Criterion	Biologic effects of ACTH and Cortisone *vs.* Salicylates	
	Similarities	Differences
Sedimentation rate (Rheumatic fever and rheumatoid arthritis)	ACTH and CORTISONE—Cause significant decrease in sedimentation rate. SALICYLATES (Schaeffer, *et al.: Circulation* **2:** 265, 1950.) Salicylates cause significant decrease in sedimentation rate in acute rheumatic fever. (Coburn: *Bull. Johns Hopkins Hosp.* **73:** 435, 1943.) Sedimentation rate decreases to normal in seven to ten days (40 cases). (Lichty and Hooker: *Proc. Soc. Exper. Biol. & Med.* **48:** 69, 1941.) Sedimentation rate decreases during salicylate therapy and increases when salicylates are discontinued (7 cases).	
Uric acid; increased urinary excretion	ACTH and CORTISONE (Thorn, *et al.: Tr. A. Am. Physicians* **60:** 143, 1946.) Increased urinary uric acid excretion. (Thorn, *et al.: J.A.M.A.* **137:** 1005, 1948.) Increased urinary uric acid excretion. (Goodman and Gilman: Pharmacological Basis of Therapeutics, page 227, 1941.) Therapeutic doses of salicylates increase the urinary excretion of uric acid.	
Catabolic effect	ACTH and CORTISONE—The catabolic effect of ACTH and cortisone is well established. SALICYLATES (Reid, *et al.: Quart. J. Med.* **19:** 1, 1951.) Salicylates cause negative nitrogen balance.	
Hyaluronidase	ACTH and CORTISONE (Benditt, *et al.: Proc. Soc. Exper. Biol. & Med.* **75:** 782, 1950.) ACTH and cortisone inhibit increase in capillary permeability caused by hyaluronidase. (Opsahl: *Yale J. Biol. & Med.* **22:** 115, 1949.) Cortisone inhibits spreading phenomenon. SALICYLATES (Guerra: *Science* **103:** 686, 1946.) Salicylates inhibit by 60% the spreading effect of hyaluronidase in rabbits. (Swyer: *Biochem. J.* **42:** 32, 1948.) Salicylates have no effect on hyaluronidase *in vitro*. (Perhaps act through pituitary-adrenal system.)	
Skin sensitivity reactions	ACTH and CORTISONE (Germuth: *Proc. Soc. Exper. Biol. & Med.* **76:** 177, 1951.) ACTH and cortisone suppress Arthus' phenomenon in rabbits. (Bradley and Cutting: *Bull. Johns Hopkins Hosp.* **87:** 186, 1950.) ACTH and cortisone reduce reaction to tuberculin (human subjects). SALICYLATES (Fischel: *Proc. Soc. Exper. Biol. & Med.* **66:** 537, 1947.) Salicylates decrease edema of Arthus' phenomenon. (Schwartzmann: *Proc. Soc. Exper. Biol. & Med.* **75:** 175, 1950.) Cortisone, ACTH and salicylates inhibit tissue reactivity to bacterial antigen in rabbits.	

TABLE 1—*Continued*

Criterion	Biologic effects of ACTH and Cortisone *vs.* Salicylates	
	Similarities	Differences
Serum vascular disease	ACTH and CORTISONE (Rich, *et al.: Bull. Johns Hopkins Hosp.* **87:** 549, 1950.) Cortisone inhibits S.V.D. (Seifter, *et al.: Proc. Soc. Exper. Biol. & Med.* **75:** 337, 1950.) Cortisone inhibits S.V.D. SALICYLATES (MacGregor and Wood: *Brit. J. Pharmacol.* **5:** 9, 1950.) Salicylates inhibit S.V.D. (Smull, *et al.: J. Lab. & Clin. Med.* **33:** 936, 1948.) Salicylates inhibit S.V.D. (Sullivan, *et al.: Proc. Soc. Exper. Biol. & Med.* **67:** 508, 1948.) Salicylates inhibit S.V.D. (Roberts, *et al.: Arch. Int. Med.* **83:** 48, 1949.) Salicylates inhibit some aspects of S.V.D.	
Ocular inflammation	ACTH and CORTISONE Ocular anti-inflammatory effects well established. SALICYLATES (Gifford: *J.A.M.A.* **113:** 928, 1939.) Salicylates produce diminution in ocular inflammatory changes.	
Cushing's disease	ACTH and CORTISONE—Produce Cushing's syndrome. SALICYLATES (Cochran: *Brit. M. J.* **2:** 1411, 1950.) Aspirin can produce signs of mild Cushing's syndrome (1 case). Salicylates can produce acne and puffy face (other cases).	
Anaphylactic shock	ACTH and CORTISONE (Nelson: *Proc. Soc. Exper. Biol. & Med.* **75:** 181, 1950.) Cortisone reduces mortality from anaphylactic shock in mice. (Selye: *Canad. M.A.J.* **61:** 553, 1949.) ACTH and cortisone inhibit anaphylactoid reaction. SALICYLATES (Lepper, *et al.: Proc. Soc. Exper. Biol. & Med.* **74:** 254, 1950.) Salicylates decrease incidence of death from anaphylactic shock in rabbits. (Campbell: *Science* **108:** 478, 1948.) Aspirin protects rabbits against anaphylactic shock.	ACTH and CORTISONE (Dworetzky, *et al.: Proc. Soc. Exper. Biol. & Med.* **75:** 201, 1950.) Have no effect in guinea pigs. (Landau, *et al.: Bull. Johns Hopkins Hosp.* **88:** 395, 1951.) Have no effect.

Endocrine Review

DEGENERATIVE COMPLICATIONS OF DIABETES: A REVIEW

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PREVENTION and treatment of premature degenerative complications is today the most important problem in the management of diabetes mellitus. As long as treatment with diet and insulin is adequate to prevent loss of weight, polyuria, acidosis, and other annoying symptoms, patients may do well for several years. It is the development of generalized arteriosclerosis, retinal lesions, diabetic nephropathy and neuritides after ten to twenty years of diabetes, which presents a distressing sequence of the disease, particularly in patients between 30 and 50 years of age.

In recent years, premature vascular disease with its manifestations in the retina, the coronary arteries, the kidneys and the peripheral vessels has been regarded as related inevitably to the duration of diabetes and possibly to unidentified "X" factors. Some writers (Dolger (1), Lichtenstein (2), and Tolstoi (3)) have therefore endorsed the program of management in which a free or unmeasured diet is allowed and insulin given daily to prevent acidosis and actual symptoms. Opposed to this view have been many authors who believe that every effort should be made to restore physiologic conditions, including freedom from glycosuria, and to secure a normal blood sugar level as far as it is practicable. Baeser (4) found in an analysis of the replies to a questionnaire sent to 1 out of 3 members of the American Diabetes Association that among 225 replies, 70 per cent of the physicians aimed at obtaining normal blood sugar levels, 17 per cent aimed at aglycosuria before meals, 6 per cent at aglycosuria after meals, 4 per cent accepted mild glycosuria and only 3 per cent disregarded glycosuria. Proponents of consistent vigorous treatment believe that the degenerative complications are related directly to inadequate control of diabetes (Joslin *et al.* (5), Wilson, Root and Marble (6), and Jackson *et al.* (7)). Obviously the demonstration of the specific causative mechanisms, (infectious, metabolic, nutritional or endocrine) will depend upon further investigation both in the laboratory and the clinic.

This review is limited to four of the major degenerative sequelae. The other protean complications of diabetes, such as gangrene, and necrobiosis lipoidica diabeticorum, are described by Joslin, Root, White and Marble (8).

VASCULAR DISEASE

A shift in emphasis has occurred with regard to vascular disease in diabetes. Although sclerosis of the larger arteries is still important, it is the characteristic lesions in smaller blood vessels (arterioles, venules and capillaries) which seem specific for diabetes.

Usually three distinct arterial degenerative processes are grouped together, as being especially common in the diabetic population, (*a*) atherosclerosis, (*b*) calcification of the arterial walls, chiefly of the media (Monckeberg's sclerosis), and (*c*) arteriolarsclerosis. All grades of involvement are found, from minimal to extensive. They are apparently independent of each other and may occur singly or in combination. The co-existence of calcified arteries, arteriolarsclerosis and retinal lesions is of special importance in cases of pregnancy in young women with diabetes of long duration. Priscilla White (9) and Nelson, White and Gillespie (10) have stressed the importance in obstetric management and in prognosis of a classification of diabetic pregnancies based chiefly upon the presence and extent of these measurable abnormalities. In their experience with diabetes of long duration calcified pelvic arteries are often associated with small-vessel change, ovarian hypofunction and an uncertain prognosis, unless adequate hormonal therapy is used.

The frequency of increased capillary fragility (Rodriguez and Root ((11) and Barnes (12)) as an early evidence of impending vascular degeneration, and notably its association with retinal hemorrhages in young diabetics and capillary aneurysms in the retina (Ballantyne (13, 14), Friedenwald (15), Warren and LeCompte (16)), give a new place to the capillaries in discussion of vascular complications. The venous lesions in diabetic retinitis make complete the involvement of the various elements of the vascular system. Whatever the underlying causes may be, all these anatomic elements may be affected in diabetes, especially if the disease is not well controlled and of long duration.

Although atherosclerosis may be the most common and clinically important change in diabetes as a whole, it is the hyaline change in arteriolar walls with arteriolarsclerosis which is both characteristic and malignant in younger diabetics in whom the disease is of long duration. E. T. Bell (17) reported an analysis of postmortem examinations upon 1,214 individuals who had diabetes at the time of death, among a total of 49,922 postmortem examinations between 1910 and 1948 in the Laboratory of Pathology at the University of Minnesota. Vascular complications as a cause of death rose from 17 per cent in the years 1910–1922 to 49.6 per cent in the years 1936–1948. Duration of diabetes was an important factor in the vascular complications of Bell's series, in patients up to age 60 years. Coronary disease was twice as frequent in the diabetics as in the controls. Renal

arteriolarsclerosis of grade 3, meaning severe change in the afferent glomerular arterioles was 100 times as frequent in the diabetics as in the controls. Among 157 autopsies at the New England Deaconess Hospital, upon patients with diabetes for fifteen or more years analyzed by Warren and LeCompte (16), coronary atherosclerosis caused death in 43 per cent. Atherosclerosis, especially involving coronary and peripheral arteries, remains the chief cause of death in diabetes as a whole.

Atherosclerosis is defined in descriptive morphologic terms in the absence of a clear understanding of its etiology, according to Duff and McMillan (18). The word indicates a pathologic change affecting the intima of arteries, characterized by focal thickenings of the intima in which stainable lipids can readily be demonstrated in and between cellular elements. The lesions show, at one extreme, an almost entirely fibrous composition with only minimal quantities of lipid material, whereas at the other extreme they contain lipid accumulations of massive proportions with extensive necrosis of the central parts of the lesions commonly culminating in disruption of the intimal lining. The lesions are highly variable and present all gradations. Atherosclerosis begins in childhood and is almost invariably present after adolescence in minimal degrees, indicating an inherent susceptibility of the human species to this disease. The fact that the lesions are characteristically patchy indicates the operation of local factors. Nearby areas of the intima may remain entirely normal. A most striking feature is the great accumulation of cholesterol and other lipids in the lesions. The quantity is so great that it is highly conceivable that the lipids originate from local destruction of tissue, although it is generally accepted that lipids infiltrate the intima from the blood plasma. This process is accelerated by the presence of hypercholesterolemia. More detailed discussions are available in the Proceedings of the American Society for the Study of Arteriosclerosis (19), in the Seminar on Degenerative Lesions of Metabolism of the National Institute of Health (20), in Anitschkow's (21) chapter on experimental arteriosclerosis, and in the third edition of Warren and LeCompte's Pathology of Diabetes (16).

Instability of the solution of cholesterol in the blood, rather than the level of increased cholesterol concentration itself, is the general condition required for its deposition. This stability of the cholesterol in the blood plasma depends largely on its relation to other substances, among which the phospholipids have been given special importance as stabilizing agents. The rapid development of atherosclerosis is associated with diseases commonly accompanied by increased cholesterol concentrations in the blood. However, an increase in the cholesterol level of the blood is lacking in the great majority of patients who show the typical deposits. In addition to plasma concentration of cholesterol and other lipids, the number and size

of chylomicrons in the plasma, a tissue factor such as chondroitin sulfuric acid or other carbohydrate sulfate esters in the arterial intima, and the blood pressure are to be considered, according to Duncan (22). The recent use of the analytic ultracentrifuge in identifying giant lipoprotein molecules (Gofman *et al.* (23)) in the sera of patients and of cholesterol-fed rabbits has provided a newer and more exact measurement than any other method. Gofman *et al.* found that it is not the plasma cholesterol itself which is related to coronary vascular sclerosis but the presence and quantity of a particular group of lipoproteins which contain cholesterol. Preliminary data suggest that dietary restriction of cholesterol may reduce the amount of this lipoprotein.

Keys (24, 25) analyzed the data supplied by Gofman in 89 healthy men and in 98 men with coronary disease. It is noteworthy that the normal subjects were younger in average age than the patients with coronary disease. Applying statistical analysis to the data, Keys concluded that there was substantial correlation between the incidence of coronary disease and the concentrations in human blood serum of total cholesterol and of the "giant molecules" characterized in the ultracentrifuge by the -10 to -20 Svedberg units of sedimentation. A similar correlation was found in the concentrations of these lipoproteins and the cholesterol. However, he found no evidence that the concentration of the lipoprotein was of greater value than the level of total cholesterol in detecting or predicting coronary disease.

Morrison and Gonzales (26) reported that the administration of large doses of choline significantly reduced the mortality rate due to recurring coronary thrombosis in a series of 115 patients with coronary arteriosclerosis. Further reports on this important series of patients should be anticipated. The use of choline may seem superfluous, since the diet normally contains much choline, as Best has emphasized. The use of inositol and other lipotropic substances is being studied. Moreton (27) suggested that in normal persons with normal plasma lipid levels the development of arteriosclerosis may be attributed to the large particle size of recently absorbed lipids called chylomicrons, which appear after a rich fat meal. The hypothesis is that large lipid particles are entrapped in the intima and stimulate phagocytosis by macrophages with the formation of "foam" cells. Local conditions in the walls of the arteries are difficult to assess. The nature of these various local factors and their relative importance has not been clearly defined. Apparently damage of a wide variety to the arterial wall may lead to the development of atherosclerosis. Little is known of the means by which lipids enter the arteries or how these vessels become thickened.

The objectives in treatment are to restore the disturbed physiologic processes to normal as nearly as is possible (Duncan (22) and Barach (28))

and thus provide relief from, and reduce as much as possible the symptoms and clinical evidences of the disease. This is in effect the control of diabetes. Hyperglycemia, hypercholesterolemia and glycosuria should be prevented by the suitable adjustment of diet and insulin. Changes in diet and insulin dosage should be made more gradually and conservatively in patients who have far advanced vascular disease than in those with minimal or no evident changes.

Hypercholesterolemia, excessive cholesterol esters and lipemia are frequently recognized in stages of diabetes when the condition has been uncontrolled or when conditions affecting metabolism and intracellular utilization of life-giving factors are disturbed. The high incidence of coronary arteriosclerosis in patients who have xanthomatosis is well known. Alterations in lipid transport and lipid metabolism are best accomplished (*a*) when obesity is corrected and (*b*) when a diet of moderately high carbohydrate intake is not only taken, but its normal utilization made possible by an adequate dosage of insulin. A diet containing from 175 to 250 grams of carbohydrate is often desirable as a means of reducing high blood lipid values. The fat components of the diet may be maintained at 65 to 90 grams, as is necessary to produce a total caloric content which will make possible the reduction of obesity to normal body weight.

Despite past controversies, evidence exists that even in normal individuals serum cholesterol concentration will fall if the intake of fat is sufficiently restricted (Mellinkoff *et al.* (29) and Watkin *et al.* (30). Keys (31) studied 482 "normal" males with diets varying in cholesterol content from 250 to 800 mg. daily, and 41 males on a low-cholesterol diet, for at least one year. He concluded that the serum cholesterol level is independent of intake over a wide range, but with no cholesterol intake the blood level falls at a rate related to the previous blood level. Hildreth *et al.* (32) describe results obtained with 3 young physicians who reduced the fat in their diets to daily amounts of 9, 10 and 62 grams for periods of from three to eight months. The levels of plasma cholesterol declined, but rose again when vegetable fat was added to the diet. The restriction of total fat and not of cholesterol alone was probably the decisive factor in lowering the serum cholesterol concentration. Although very low fat diets are needed to lower serum cholesterol in nondiabetics, only moderate restriction of fat in the diet, combined with the use of insulin, rapidly lowers high serum cholesterol values when due to uncontrolled diabetes.

The important place of protein is receiving new emphasis and the former low-protein diets have been largely discontinued. Adult diabetic patients, who are physically active, usually require from 85 to 120 grams of protein a day or approximately 1 gram to $1\frac{1}{2}$ grams per kilogram of body weight. A growing child may require from 2 to 4 grams per kilogram of body weight.

The correction of obesity will tend to prevent as well as to reduce the progress of degenerative arterial changes. Circulatory failure is more common in the overweight than in individuals of normal weight. Wilens (33) has shown that a reduction in body weight may favor regression of atheromatous changes and a reduction in the lipid content of the plaques. A 50-year-old thin subject may have less atherosclerosis than a 40-year-old fat person.

NEUROPATHIES

Degenerative changes in the nervous system play an important role in diabetes of long duration, yet demonstrable changes in the nervous system are largely due to complications of the disease, such as arteriosclerosis or hypoglycemia, rather than to diabetes itself. The question as to the influence of the central nervous system in the etiology of diabetes is not involved in the present discussion. Degenerative changes consequent upon diabetes may involve the higher centers of the brain, the spinal cord, the sympathetic system, or the peripheral nerves. Griggs and Olsen (34) described three types of cord involvement as occurring in diabetes: (*a*) degeneration of the motor cells of the brain stem and spinal cord; (*b*) degeneration of the intramedullary portion of the dorsal root fibers with secondary system degeneration, producing a lesion like tabes dorsalis; and (*c*) funicular necrosis of the posterior columns, occurring especially in the cervical and dorsal segments of the cord. The significance of these lesions and their specificity may be questioned, according to Warren and LeCompte (16). Changes accompanying the acute hypoglycemic state are not discussed here, although it is true that hypoglycemic damage of brain centers may leave permanent alterations with consequent mental changes of the most serious nature. Collens (35) and his co-workers found neurologic abnormalities in 93 per cent of all their diabetic patients. The cause (or causes) of the disturbances is not entirely clear because some workers have recorded the presence of these early lesions in well treated patients and in some cases before the actual recognition of diabetes. The fact remains, however, that the more severe types of diabetic neuropathies are seen almost entirely in patients whose diabetes has not been under adequate control for considerable periods of time. Duncan (22) considers that there may be an underlying disturbance which explains neuropathies seen in patients with mild diabetes under good control; this disturbances tends to be intensified by poor control of the diabetes and particularly by loss of weight. Its great frequency in diabetic patients compared to nondiabetic patients, however, leaves a basic conviction that diabetes itself in some way has a direct bearing upon the etiology.

Disturbances in vibration sense are recognized clinically by diminished

acuity or inability to recognize the vibrations of a tuning fork or by the increased perception time. Barach (36) and Collens (35) have made extended studies of this method of examination. There can be no doubt that diminished sensation is found in diabetic patients without disturbance of blood supply and that the lesion is hardly likely to be primarily due to diminished blood supply. It is true, however, that in patients who have arteriosclerosis and particularly lesions in the vessels accompanying the nerve trunks themselves, disturbances in nerve function do occur. For the study of impaired vibration sense, a tuning fork recommended by Barach is perhaps superior, but smaller instruments may be used. The degenerative changes which account for the blocking of the transmission of vibratory impulses occur in the posterior columns of the spinal cord and in the peripheral nerves. Barach followed 150 patients for periods up to fifteen years. In some cases there was definite improvement under treatment. The striking feature of Barach's results was the demonstration of curves approaching normal or receding from normal corresponding with improvement or aggravation of the diabetic status. In the studies of diabetic neuropathy at the New England Deaconess Hospital (37) the finding of increased protein in the cerebrospinal fluid in amounts varying from 50 mg. to 300 mg. per 100 cc. was roughly correlated with the severity and the duration of neuropathy, suggesting involvement of the central nervous system rather than of the peripheral nerves alone. Changes of a chemical nature in the nerves themselves are demonstrable, but the response of patients in early stages of typical diabetic neuropathy to control and regulation of the diabetes by insulin, diet and adjunct vitamins, is consistent with the concept that the lesions are chiefly of the central nervous system rather than of the peripheral nerves alone.

One may distinguish between the chronic neuropathies and acute neuropathies of diabetes. In the early or mild forms of neuropathies the symptoms are often generalized, beginning with vague aches and pains in the arms, trunk and legs, with various paresthesias which are particularly marked at night and upon exposure to cold. Relief is obtained by walking, in contrast to pains due to arthritis or impaired blood supply, which are made worse by exertion. The onset of neuropathy after diabetes is brought under control has been reported. Careful questioning usually reveals symptoms at an earlier period before treatment. It is only rarely that increased carbohydrate utilization resulting in thiamine deficiency, as can be considered a cause of neuropathy.

Severe and refractive alterations include permanent loss of the knee jerks and Achilles reflexes, reduced pupillary reflexes and even Argyll-Robertson pupils, which were described by Waite and Beetham and are referred to by Duncan (22).

Neurotrophic feet or pseudo-Charcot joints were described by Bailey and Root (38) in a series of 20 patients presenting a painless deformity of the feet, chiefly in the tarsus; but destruction of knee points has been observed by Rundles (39). Chronic ulcerations of the feet may accompany these lesions. The bony changes are not due to infection, but show a structure similar to that of the Charcot joint.

Two patients, aged 31 years and 42 years respectively, with typical neuropathic lesions in the tarsus are reported by Parsons and Norton (40). Each had evidence of peripheral neuritis and the lesions were associated with infections in the toes. Lumbar sympathectomy was performed in an effort to arrest the destructive process, and in the hope of improving the blood supply. At the end of twenty-eight and forty-seven months respectively, radiographic and clinical evidence justified the belief that no progression had occurred.

A comprehensive summary of diabetic neuropathy by Rundles (39) emphasizes its frequency and variety in approximately 5 per cent of all diabetics. In diabetic neuropathy, pain and thermal paresthesias are prominent symptoms. Diabetes produces to a greater extent than any other disease a profound disturbance in the function of the autonomic nerves. Pain and thermal sensibilities and autonomic functions are mediated by small-caliber, poorly myelinated neurons. In pernicious anemia, on the other hand, the large-caliber heavily myelinated neurons conducting motion, position and vibratory sensibilities are affected early and collectively, and pain is rarely an important complaint. In pernicious anemia, degeneration of the lateral and posterior columns of the spinal cord leads to exaggerated tendon reflexes, clonus and spasticity, which types of nervous disorder do not occur in diabetes; nor do the symptoms of cerebral involvement, such as confusion, depression and psychosis. Loss of vibration sense is common in both diseases. In diabetes, bizarre manifestations occur during a neuropathic state, such as night sweats (sometimes limited to the upper third of the body), intolerance to heat and cold, tachycardia and genito-urinary and gastro-intestinal complaints. As a result of orthostatic hypotension, dizziness and black-outs are frequent and we have seen at the New England Deaconess Hospital fractures of the hip, skull and collar bone in patients with neuropathies. The gastro-intestinal disturbances, particularly those involving gastric motility sometimes resemble those seen after vagotomy.

During acidosis or states of poor diabetic control, pain and paresthesias are common, but rapidly subside with treatment which leads to control of the diabetes. The diagnosis of diabetic neuropathy, then, should be made only when evidences of disturbed nerve function persist well beyond any temporary period of non-regulation of the diabetes. In discussion, Foster Kennedy (39) pointed out that the term peripheral neuritis or polyneuritis

should not be applied to diabetic neuropathy. It is obvious that when sphincter abnormalities, sexual impotence and marked autonomic imbalance such as nocturnal diarrhea occur, peripheral neuritis is not the cause. Clearly these lesions are the result of inadequacy of the spinal roots, the spinal cord and of the autonomic system itself. Kennedy prefers the word neuronitis as indicating the fact that in true diabetic neuropathy it is the entire neuron or portions of the entire neuron which are involved. This is a good distinction and makes the variety of lesions more comprehensible. Milton Handelsman (39) in further discusssion gave an anatomic description by asserting that diabetic neuritis is really a radiculo-myeloneuritis. Nerve survival and action are intimately associated with an electrical potential which correlates with nerve activity. Nerve potential is due to changes in polarization along neuronal surfaces. One explanation out of many has to do with the synthesis and breakdown of acetyl-choline along the surface of the nerve. The link connecting this system with glycolytic activity is through adenosine-triphosphate and creatine phosphate. These substances supply energy to resynthesize acetyl-choline, and the utilization of carbohydrate supplies energy to resynthesize adenosine-triphosphate.

Treatment of the diabetic neuropathy. In acute stages, bed rest and control of the diabetes are essential. Sometimes it appears that repeated lumbar punctures and the use of vitamin B_{12} as advised by Duncan (22) are effective measures in correcting the neuropathic disorder. Intramuscular doses of 30 mg. are recommended. Barach has apparently had an experience similar to that at the New England Deaconess Hospital, where the use of vitamins in large amounts either by mouth, by vein or intramuscularly has seemed to be at times effective, but in many patients entirely without effect. Broch and Klovstad (41) studied in Norway 88 cases of polyneuritis, representing 20.7 per cent of one country's diabetics. No benefit was obtained from the use of vitamin B_1.

The use of BAL (British Anti-Lewisite) has been tried by Schneider (42) in the treatment of diabetic neuritis. Underlying this experiment was the realization that the effectiveness of BAL seemed to be due to its ability to supply sulfhydryl radicals. BAL (2, 3-dimercaptopropanal) is recognized as a potent compound containing two sulfhydryl radicals: these sulfhydryl radicals have a great affinity for heavy metals. Many enzyme systems contain heavy metals within their chemical structures. Many heavy metals produce toxicity because of their great affinity for sulfhydryl radicals present in the prosthetic group. The application of BAL to the treatment of arsenical intoxication was based on its ability to supply competing sulfhydryl radicals in order to protect, as well as to reactivate, previously affected enzyme systems. The reversibility of such biochemical processes is con-

firmed. It is evident from animal experimentation that the administration of heavy metals prior to the administration of BAL increases the tolerance of the animal for BAL. Toxic side effects, common in small doses in normal animals, do not result when larger doses are given. It is thus apparent that heavy metals are antidotes for BAL. The BAL and other sulfhydryl compounds are antidotes for heavy metals. It appears evident that the doses of BAL recommended for the treatment of heavy-metal intoxication cannot apply to persons with diabetes, since it has not been ascertained that the neurologic manifestations of diabetes bear any relationship to heavy-metal poisoning. However, it was held that neuropathies might represent biochemical disorders within the neurons, consisting of impairment of enzymatic function. It was decided to test the virtues of BAL. Twenty-two patients with diabetic neuropathy were treated with BAL after each had received treatment for varying periods with other methods, to which all had failed to respond. Twelve of the 22 improved greatly under treatment with BAL. There were 3 who obtained definite alleviation of pain, numbness and burning. BAL was administered intramuscularly in one dose of 50 mg. on the first day and was increased to 100 mg. on the second day. The dose generally remained at 100 mg. once or twice a day. The duration of treatment was from seven to fourteen days.

DIABETIC NEPHROPATHY

Renal lesions which complicate diabetes may be listed as follows:

1. Presumably reversible metabolic phenomena:
   (a) Glycogen deposits in tubules.
   (b) Fat deposits in tubules.
2. Arteriolarsclerosis (nephrosclerosis).
3. Intercapillary glomerulosclerosis.
4. Acute and chronic pyelonephritis, including necrotizing renal papillitis.
5. Arteriosclerosis.

Glycogen deposits in the tubules merely indicate glycosuria. Fat deposits in the tubules indicate injury to the tubular epithelium and commonly occur in coma. Arteriolarsclerosis appears earlier and is more common in diabetes than in other diseases. The last four lesions commonly occur together, especially in younger patients in whom diabetes has been of long duration. So characteristic is this picture that Wilson, Root and Marble (6) use the term diabetic nephropathy to indicate the combination of arteriolarsclerosis, intercapillary glomerulosclerosis, chronic pyelonephritis, and arteriosclerosis.

Although the frequency of vascular nephritis in middle-aged patients with diabetes of long duration was noted by Root and Warren in 1926, it was in

1936 that Kimmelstiel and Wilson (43) pointed out a correlation between a syndrome of diabetes, nephrotic edema, gross albuminuria and hypertension, and a type of intercapillary sclerosis of glomeruli. Although they did not stress the clinical symptoms and reported only a small group of patients, as time has passed the importance of this syndrome has steadily increased. At first the intercapillary glomerulosclerosis was considered to be a condition found chiefly in middle and late life, but in recent years this syndrome, ending in uremia and death, has become a most important complication in diabetes of ten or more years' duration, with onset in childhood and youth.

Kimmelstiel and Porter (44) reported an average of 17.1 per cent for the incidence of intercapillary glomerulosclerosis in six reported series, composed predominantly of patients in middle or old age. The lesion is most frequent in young people who have diabetes in severe form and of long duration. Millard and Root (45) analyzed 110 autopsies, including 12 autopsies performed on patients whose diabetes began in childhood. In this group of childhood diabetics everyone with diabetes of over ten years' duration showed Kimmelstiel-Wilson lesions. In a similar summary White and Waskow (46) stated that every one of 30 childhood diabetics dying with diabetes of over ten years' duration showed the lesions. Warren and LeCompte (16) recorded the presence of the lesion at autopsy in almost 100 per cent of their young diabetics who had had the disease for a long time.

The observations on renal pathology in intercapillary glomerulosclerosis have been confirmed by many authors, but some disagreement has arisen regarding their interpretation.

The typical lesion consists of a hyaline rounded or globular mass in the central portion of the glomerular lobules, creating the impression of being situated between the capillaries. However, this lesion has been found in nondiabetics and in arteriosclerotic kidneys in from 0.8 to 12.0 per cent of cases according to Goodof (47) and Laipply *et al.* (48). Yet Siegal and Allen (49) found typical changes in only 1 out of 100 nondiabetics (hypertensive patients) and Bell (50) considers the nodular types of intercapillary glomerulosclerosis practically pathognomonic for diabetes.

The diffuse type, according to Bell, resembles the changes seen in chronic glomerulonephritis and is formed by splitting of the inner basement membrane. It may be present alone or in conjunction with the nodular type. Indeed, Kimmelstiel and Wilson (43) linked it to a rather frequent glomerular change in senile kidneys. They called this process "axial" glomerular thickening and part of the "aging" process.

Bell states that a thick homogeneous hyaline deposit in the arterioles is strong evidence of diabetes. It seems clear that in some way diabetes

at an early stage in life brings about hyaline changes in the arteries and arterioles, with consequent hypertension and renal disease. This was particularly shown in the series of young people reported by Root (51), in which among 531 deaths of diabetic patients with onset of diabetes before the age of 30, nephritis was the cause of death in 30 per cent of the 142 patients who had had diabetes more than fifteen years at the time of death.

The hyaline deposits in the glomeruli often resemble amyloid, but most authors agree that it can not be so identified. The use of the periodic acid stain has suggested that this material and the hyaline material in retinal arterioles, the islands of Langerhans and basement membranes are related. Most observers have agreed with the original description, with the exceptions of Allen (52), Laipply (48) and Bell (53), who believe that the lesions are produced by a splitting of the capillary basement membrane and that the hyaline deposits are intracapillary rather than intercapillary. A review of experience to date indicates that the complete clinical syndrome with edema of the nephrotic type, albuminuria, hypertension and diabetes occurs in a relatively small percentage of the patients who have a pathologic picture of intercapillary glomerularsclerosis. It can be said, however, that diabetes occurs in almost all cases of intercapillary glomerulosclerosis, when the fully developed lesions can be demonstrated at autopsy.

The specificity of the hyaline masses in the glomeruli has been questioned by certain authors, notably Christian (54), but most other authors have agreed with the concept of a high degree of specificity for the fully developed lesion. Robbins and Rogers (55) reviewed 100 proven cases of intercapillary glomerulosclerosis and 229 cases of diabetes without this lesion. As a clinical entity, intercapillary glomerulosclerosis was not clearly defined and only 40 per cent of their cases could be diagnosed on clinical grounds alone. Several apparently nondiabetic cases proved on careful investigation to be really diabetic. They concluded that, in the nodular form, the lesion is a specific histologic entity occurring only in diabetics.

A striking indication of the specificity of intercapillary glomerulosclerosis has been the experimental production of the lesions by Lukens and Dohan (56) in a dog made diabetic by extract of the anterior lobe of the pituitary gland. After a five-year period a postmortem examination showed lesions resembling the early lesions of intercapillary glomerulosclerosis. More recently Mann and Goddard (57) have found in rats made diabetic with alloxan, a lesion similar to that in the human disease. They found thickening of the basement membrane followed by deposition of hyaline, acellular material between the capillary loops, with, unlike the human lesion, adhesion of the tufts to one another or to Bowman's capsule. The lesions are probably similar to those described by Foglia *et al.* (58) in rats made diabetic by partial pancreatectomy.

A close relation between intercapillary glomerulosclerosis and arteriolosclerosis in the kidney has been reported by all observers. Arteriolosclerosis can not be regarded as the sole cause of the condition. Allen (52) noted that this arteriolosclerosis of the afferent glomerular vessels was absent in nondiabetic patients. Hypertension, when it occurs in this condition, is likely to be correlated with arteriolosclerosis (59–66).

Clinically, intercapillary glomerulosclerosis and chronic glomerulonephritis may resemble each other. The absence of acute glomerulonephritis may aid in differentiation. Henderson, Sprague and Wagener (67) compared patients who had diabetes and intercapillary glomerulosclerosis with patients who had glomerulonephritis in which intercapillary lesions were present. The latter group averaged only 32 years of age, whereas the group of diabetic patients averaged 58 years of age. Edema, albuminuria and the evidences of uremia were more intense in the cases of glomerulonephritis. They concluded that intercapillary glomerulosclerosis in diabetes constituted a pathologic process different from intercapillary glomerulosclerosis accompanying chronic glomerulonephritis.

The nephrotic syndrome when it occurs in diabetic patients has so far been reported only in cases showing intercapillary glomerulosclerosis. However, in the New England Deaconess Hospital series (6), in some instances the lesions of pyelonephritis far outweighed the pathologic evidence of intercapillary glomerulosclerosis. The data in case reports concerning the low blood protein level and the high cholesterol values with edema, have been so far incomplete and variable. Newburger and Peters (68), as well as Porter and Walker (69), reported significant albuminuria, hypoproteinemia and edema in 75 to 100 per cent of their cases. Siegal and Allen (49) concurred in this view. Laipply (48), however, found the nephrotic syndrome in only 6.3 per cent of his cases and edema was present in only a third of Bell's cases. It is evident, therefore, that the pathologic lesions of this condition may be present without the nephrotic picture of edema, low blood protein, high blood cholesterol, and albuminuria in a large percentage of the cases.

Rifkin *et al.* (70) report the postmortem examinations of 22 patients with the clinical diagnosis of diabetic glomerulosclerosis. The diabetes in these patients was classified as mild when it was controlled with 0 to 10 units of insulin in a twenty-four-hour period; moderate, when the need for insulin was 11 to 30 units; and severe, when more than 30 units was required in twenty-four hours. Their youngest patient was 25 years of age and the oldest was 73, but 3 of the 22 patients were in the third decade. The report makes special reference to the presence of doubly refractile lipoid cells or casts in the urine. They were noted in 39 out of a group of 44 patients. These fatty droplets are usually present in epithelial cells, occasionally

in white cells, and frequently in the form of casts. In polarized light with crossed planes they are easily recognized. The fatty cells and casts contain bright achromatic maltese crosses with dark crosslines. These authors point out the rarity of red blood cells in the sediment. Twenty-four of their patients died of uremia. Wilens *et al.* (33), studying frozen sections stained with Sudan IV in 21 cases of the disease, found a larger amount of fat in the glomeruli than in any other of the common types of renal disease. The quantity of fat in the glomeruli was directly proportional to the severity of the lesions in most instances. An excellent analysis of the complications in a series of 88 diabetic children observed over a period of years is given by Rosenbusch (71). He noted the close relationship between retinitis and the characteristic diabetic nephropathy or intercapillary glomerulosclerosis. He maintained that the retinal hemorrhages did not follow, but rather preceded, the arteriosclerotic changes and the hypertension.

In a review of 43 cases of intercapillary glomerulosclerosis Mann, Gardner and Root (72) noted that out of 43 patients, 36 were between 20 and 30 years of age. None had had good control of diabetes, 45 per cent of the series were recorded as having poor control, and the remainder had "fair" control. All patients required insulin. Only 3 of the group required less than 20 units a day. Twenty-three required between 20 and 40 units, 15 required between 40 and 60 units daily, and 2 patients required over 60 units a day. The striking feature in this group was the early development of albuminuria, at first small in amount and transient, later becoming permanent and massive. Edema at first was not consistently related to the level of serum protein and the hypoproteinemia developed considerably later than the edema. Hypertension developed early, but not until after albuminuria had been present. It was the early onset of edema and the massive proteinuria which really distinguished this condition from the malignant form of nephrosclerosis seen in nondiabetics, Neuropathies, including postural hypotension, were noted in the New England Deaconess Hospital cases and in the 10 cases reported by Gilliland (73). Root, Story and Cortesi (74) emphasize the possibility of confusing ketosis and uremia in such young diabetics with far advanced nephropathy. In 1 such uremic patient, aged 31 years, with spontaneous hyperkaliemia and typical electrocardiographic changes, the result was fatal. At autopsy, Kimmelstiel lesions in the glomeruli, chronic pyelonephritis, marked arteriolarsclerosis, calcified coronary arteries and some evidence of glomerulonephritis were found. Kimmelstiel and Porter (44) felt that hypertension was related to the vascular rather than to the glomerular changes. Renal dysfunction, uremia and anemia are common occurrences during the course of this condition and frequently cause of death. However, coronary occlusion and intercurrent infections have also been common causes of death. Death in uremia

occurred in 17.7 per cent of Laipply's cases. The prognosis in the younger patients has been bad. Spuhler (75, 76) and Auroi (77) predicted that death may be expected within two or three years after the syndrome has fully developed. The relationship of intercapillary glomerulosclerosis to the islands of Langerhans appears definite. Goodof (47) reported the consistent increase in the severity of diabetes after six years from the time of onset of renal disease. Practically in all cases, the diabetes occurred before the renal disease; and indeed the severity of the intercapillary glomerulosclerosis increased with the known duration of diabetes.

A frequent statement is that the lesion is unrelated to the severity of the diabetes. Measurements of severity of diabetes usually depend upon insulin dose requirements. One characteristic feature, sometimes neglected, is that as renal failure advances insulin requirements rapidly fall. Certain patients of our group at the New England Deaconess Hospital, who formerly required large amounts of insulin, have ceased to require any insulin as the renal lesion advanced. Actually the great frequency of intercapillary glomerulosclerosis in young diabetics and its development only after a considerable duration of the diabetes indicate that the condition is fundamentally related to the severity of the diabetes as well as to the duration and the degree of its control.

Evidence is now accumulating that renal lesions of this type may be prevented or postponed by adequate diabetic control with diet and insulin (Wilson, Root and Marble (6); Root, Sinden and Zanca (78)). Furthermore, Joslin reports (5) a group of 29 "medal" cases with no evidence of albuminuria, hypertension, retinal hemorrhages, or arteriosclerosis by x-ray examination, after twenty-five years of diabetes. This group consists of patients who have been under strikingly better control than average.

RETINITIS AND RETINITIS PROLIFERANS

It is the excessive frequency of retinal lesions in diabetic patients that constitutes the major and long-debated problem concerning the diabetic eye. Other lesions such as swelling and vacuolation of the pigment epithelium of the iris have long been known and the frequency of cataract in the juvenile diabetic and its early development in experimental alloxan diabetes (Waters (79)) leave no doubt of the specific influence of the disease. In many patients who have been diabetic for a long time, often without other evidence of arterial or arteriolar disease, small punctate and irregular hemorrhages develop, associated with dilatation and tortuosity of the retinal veins together with hard, white exudates. In many patients a further stage occurs characterized by many newly formed vessels and the growth of connective tissue membrane from the retina into the vitreous.

Together with this lesion a protein-rich exudate occurs and increased tension and hemorrhagic glaucoma may eventually develop. In recent years Ballantyne (13, 14) has demonstrated that some lesions originally thought to be hemorrhages are in actual fact microaneurysms. Our knowledge of these capillary aneurysms has been advanced by Friedenwald's (15) application of the periodic acid-Schiff reagent method of Hotchkiss (80) and McManus (81) to the whole mounts of the retina. This method has also proved valuable in studying the renal glomerulus, according to Warren and LeCompte (16). When the whole retina is stained and mounted, beautiful delineation of the capillary network is obtained, which shows clearly that the lesions described by Ballantyne are indeed aneurysms occurring in clumps and often with thickened hyaline walls. Friedenwald (15) and also Ashton (82) refer to the well known association of diabetic retinitis with intercapillary glomerulosclerosis. Friedenwald believes that both may represent a disturbance in the metabolism of the mucopolysaccharides. The association of these lesions is evident not only to the pathologist but also to the clinicians, as was clearly shown in the series of 326 patients with retinitis proliferans summarized by Root (83). There were 124 deaths in this group; 66 patients died of nephritis and 37 of cerebral or coronary arteriosclerosis, associated with nephritis in most instances.

The progression of retinitis during pregnancy is discussed by Beetham (84), who reported a study of 200 pregnant women. Retinitis progressed chiefly in women who already had retinitis at the beginning of pregnancy. Although advancing retinitis was in some cases a sufficient reason for terminating pregnancy, many were doing well and experienced no progression of retinitis five or more years after pregnancy had ended.

Treatment and prevention of retinitis has been a debated subject. Some authors have regarded premature vascular disease and retinitis as inevitable due to the duration of the disease, and have advocated a "free diet" and insulin given only to the extent of preventing acidosis and actual symptoms (Dolger (1); Lichtenstein (2); and Tolstoi (3)). Jackson *et al.* (7) and Wilson, Root and Marble (6) in a recent report, give evidence to support the view that severe retinal lesions can be prevented or postponed by consistent, vigorous treatment designed to provide an adequate diet and control of the diabetes by means of insulin, so that tests show the blood and urine to be normal at least a part of each day. In Wilson, Root and Marble's series from the George F. Baker Clinic, New England Deaconess Hospital, among 247 patients whose diabetes began between the ages of 18 months and 30 years and had continued for from ten to thirty-four years, a comparison of the vascular status and the retinae was made during varying degrees of control of the diabetes. The following facts appeared:

1. No patients with Excellent or Good control showed advanced retinitis or calcification of arteries, even after periods of twenty to thirty-four years.

2. No cases of diabetic nephropathy developed in patients with Good or Excellent control for periods from twenty to thirty-four years. In 62 patients maintained under Poor or Fair control, nephropathy developed. Thirteen of these patients died during the year of the study.

3. In 89 patients no retinal hemorrhages were found. This group included 35 patients with diabetes of more than twenty years' duration.

4. Fifty-five of 80 patients who lived for at least twenty years (under Poor or Fair control) on an unmeasured diet without constant use of insulin, and without control of glycosuria, showed severe degenerative lesions (calcified arteries, retinitis or nephropathy).

5. Whatever the specific etiologic factors causing diabetic degenerative lesions may be, regulation of diabetes controls these factors and thus regulation becomes more important than duration of diabetes in their prevention.

DISCUSSION

This review of four of the major degenerative complications of diabetes, necessarily omitting reference to many of the protean complications of the disease, must refer to some of the recent hypotheses advanced in an attempt to find some underlying explanation. The immediate cause of diabetes is insulin deficiency, whether due to lessened production by the islets, to antagonistic action of a hyperglycemic factor secreted by the alpha cells, to neutralization by secretions of other glands, to destruction of insulin by hypothetical substances, to failure of insulin transport, or to increased demand for insulin. Warren and LeCompte point to the concept elaborated by Friedenwald in relation to the capillary lesions of the eye and kidney. Friedenwald suggested, as did Ashton, that the staining reactions of the hyaline deposits in both diseases were similar and quoted McManus that the hyaline of the renal lesion might represent a polysaccharide deposited from the circulating blood. The animal polysaccharides may be classified as mucopolysaccharides, mucoids or glycoproteins, all of which contain hexosamine. Day (85) found that the basement membranes of ocular tissue were stained intensely by the Hotchkiss-Schiff reagent, suggesting the presence of polysaccharides. Glucosamine is thought to occur in blood in the form of a polysaccharide composed of acetylglucosamine, mannose and galactose, bound to a protein molecule of the plasma; the whole complex is termed a mucoid, or a seromucoid (Meyer (86)).

Jacobs (87) made 378 determinations of the glucosamine in the blood of 250 subjects. He found that glucosamine followed the blood sugar level and

that alterations in the blood sugar level induced by insulin were accompanied by a parallel fall in the level of blood glucosamine. Interpretation of these results is difficult because of the possibility that a certain amount of glucose is determined at the same time. With further improvements in methods, future studies in this difficult field may yield additional supporting evidence.

Warren and LeCompte carry Friedenwald's concept further with the suggestion that most of the characteristic changes, including the hydropic change and hyalinization of the islets of Langerhans, may be interpreted in terms of a disturbance in the metabolism of a simple polysaccharide (glycogen) or of the more complex carbohydrates known as mucopolysaccharides. Hyalinization of the islets may be due to the deposit of a polysaccharide-protein complex from the blood stream. These authors think that no adequate evaluation of the vascular lesions in diabetes may be made without considering the properties of the gound substance and of basement membrane. Recent studies have indicated that this ground substance of connective tissue and the component of basement membrane may be related substances containing glycoproteins, which infiltrate and are organized on a submicroscopic level. It may be recalled that swelling of the ground substance of the intima was an integral part of the inhibition theory of Virchow. Warren and LeCompte conclude that Friedenwald's concept may be extended to all major pathologic lesions characteristic of diabetes in the sense that they involve simple polysaccharides or polysaccharide complexes.

SUMMARY

1. A review of present concepts regarding the nature, treatment, and prevention of certain major degenerative complications of diabetes mellitus is given.

2. Among vascular lesions, although atherosclerosis remains the predominant cause of death, it is the lesions in the arterioles, in the venules of the retina and in the capillaries which seem equally specific for diabetes.

3. The neuropathies of diabetes involve the autonomic and central nervous systems rather than peripheral nerves alone.

4. Diabetic nephropathy, including the Kimmelstiel-Wilson syndrome, and diabetic retinopathy are directly related to the severity, duration and control of diabetes.

5. A new pathologic concept would relate the hyaline changes in the walls of capillaries and arterioles, in the renal glomeruli and in the islands of Langerhans to disturbances in metabolism of polysaccharides, or polysaccharide complexes.

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Letter to the Editor

THE CIRCULATING EOSINOPHIL RESPONSE TO EPINEPHRINE IN MONGOLISM*

To the Editor:

One of the more interesting theories concerning the etiology of mongolism places the responsibility for the condition on a defect in pituitary function. No proof has been offered for this theory, other than autopsy findings. It therefore seemed worthwhile to investigate the eosinophil response of mongoloids to epinephrine, since this test offers a physiologic rather than a pathologic criterion for the adequacy of pituitary function, at least as far as ACTH release is concerned.

The tests were carried out on a group of mongoloid subjects consisting of 12 females (ages: 7–44 years), and 11 males (ages: 6–31 years). The results were compared with the responses of a group of high-grade feeble-minded, "normal" controls, consisting of 20 females (ages: 8–30 years) and 4 males (ages: 15–27 years). A group of 5 female cretins was also studied. All subjects were inmates of a home for the feeble-minded in Columbus.

All tests were carried out on blood obtained by finger puncture, with the subject in the fasting state. The dose of epinephrine was either 0.10 cc. or 0.20 cc. of 1/1000 adrenalin hydrochloride administered subcutaneously. Blood samples were drawn and the eosinophils counted at hourly intervals up to and including four hours after the time of injection. The counts were made as described by Thorn, Forsham and Emerson (1), with some slight modifications. Two chambers of a Spencer Brite-Line hemocytometer (0.10 mm. depth) were filled with the diluted blood, and all the cells in the nine large squares in each chamber were counted. The eosinophil content per cu. mm. of blood was calculated by multiplying each chamber's content by 11.1 and averaging the resulting values for the two chambers. The initial and the four-hour blood counts were made from two pipette samples (count made from four chambers), and the intervening counts were made from single pipette samples (counts made from two chambers).

The results are shown in Figure 1, and indicate that mongoloids do not differ appreciably from "normal" individuals in their response to epinephrine. There is an apparent greater sensitivity of the mongoloids than of the normal subjects or the cretins to epinephrine; but it is doubtful, considering

* Presented by title at the Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, N. J., June 7–9, 1951.

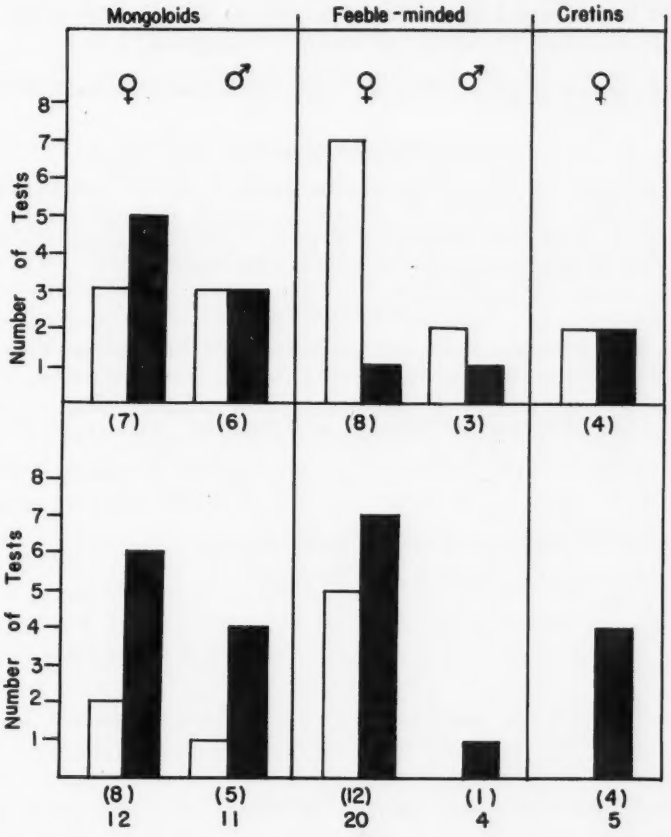

FIG. 1. *The circulating eosinophil response to epinephrine in mongolism, in feeble-mindedness, and in cretinism.* The upper portion of the figure represents the response to 0.10 mg.; and the lower portion, to 0.20 mg. of epinephrine. Decreases in the eosinophil count of less than 50 per cent are represented by open columns; and decreases of 50 per cent or more, by dark columns. On the abscissae, the numerals in parentheses represent the number of subjects in each dose group; and the numerals not parenthesized represent the total number of subjects in each diagnosis group. It will be noted that one subject served for only one test, except in the female mongoloid and cretin groups.

the number of subjects involved, if this is more than an artifact. At any rate the results justify the assumption that no real difference in response is present between normal and mongoloid individuals.

These tests certainly do not establish complete normality of the pituitary or of the pituitary-adrenal axis in mongolism. So far as they go, however, they establish the fact that the mongoloid, contrary to Benda's theory (2), does not suffer from generalized pituitary failure but possesses a pituitary

that is at least normal in its ability to release ACTH in response to an adrenergic stimulus. The extent to which the mongoloid suffers from other forms of pituitary deficiency, or from other endocrinopathies will remain for further work to elucidate.

Acknowledgment

We are indebted to Dr. Roger Gove, superintendent of the Columbus State School, for cooperating with us in this project. We also wish to thank Dr. Richard Fulton for making available to us a detailed list of the mongoloid population of the Columbus State School; and Betty Greene and Lenore Orfirer for their technical assistance.

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The Endocrine Society

34TH ANNUAL MEETING

JUNE 5-6-7, 1952

Headquarters: Palmer House, Chicago, Illinois.

Registration: Everyone attending the meetings is required to register. By action of the Council, non-members will be charged a registration fee of $5 for any part of the scientific sessions. Members should present their current membership cards when registering. Admission will be by badge only.

The Scientific Sessions: All scientific sessions will be held in the Red Lacquer Room. Sessions will begin promptly on schedule. Presentation of papers at all sessions will be LIMITED TO TEN MINUTES and authors are requested to adhere strictly to this schedule. Manuscripts of all papers should be submitted to the presiding officer or to the Secretary-Treasurer at the end of the presentation.

Annual Dinner: The Annual Dinner of the Society will be held on Friday evening, June 6, at 7:30 o'clock in the Red Lacquer Room, preceded by cocktails at 6:30 P.M. in the same room. Secure tickets at time of registration.

Local Arrangements: Dr. Willard O. Thompson, 700 North Michigan Avenue, Chicago, Illinois, is in charge of the local arrangements for the meetings.

Secretary-Treasurer: Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma.

PROGRAM

THURSDAY, JUNE 5, 1952

8:30 A.M. Registration (*Red Lacquer Room*)

I. 9:30 A.M. GONADS—REPRODUCTION (*Red Lacquer Room*)

Chairmen: Allan T. Kenyon and Samuel Soskin

1. THE BIOASSAY OF PITUITARY INTERSTITIAL CELL STIMULATING HORMONE (ICSH) IN HUMAN URINE: PRELIMINARY REPORT. *Janet W. McArthur.*

2. GONADOTROPHIN SUPPRESSION AS A RESULTANT OF THE CONVERSION OF ANDROGEN TO ESTROGEN. *C. Alvin Paulsen* (introduced by *Carl G. Heller*).

3. GYNECOMASTIA AND RETARDED SEXUAL DEVELOPMENT RESULTING FROM A LONG-STANDING ESTROGEN SECRETING ADRENAL TUMOR. *Richard L. Landau, Dwight E. Clark* (by invitation) and *Benjamin Stimmel.*

4. The Isolation and Identification of Testosterone, 4-Androstenedione-3,17, and 7-Ketocholesterol from Spermatic Vein Blood. *C. D. West* (by invitation), *V. P. Hollander* (by invitation), *T. H. Kritchevsky* (by invitation) and *K. Dobriner.*

5. The Use of Spleen Glucuronidase as a Hydrolytic Agent for Pregnanediol Assays. *Saul L. Cohen* and *M. M. Goldfine* (introduced by *Dr. G. T. Evans*).

6. The Isolation of Progesterone from Human Placenta. *William H. Pearlman* and *Emily Cerceo* (by invitation).

7. High Progestational Activity of 19-Norprogesterone. *William W. Tullner* (by invitation) and *Roy Hertz.*

8. New Ways to Increase Progesterone Activity. *Paul Engel, Carlos M. Garcia, V. Siegfried Fischl* and *Aldo Muggia* (introduced by *Max Goldzieher*).

9. Serum and Urinary Beta-Glucuronidase Levels in Patients with Prostatic Cancer. *F. T. Brayer* (by invitation) and *J. B. Trunnell.*

10. The Production of Hypertension, Nephrosclerosis and Cardiac Lesions by Methylandrostenediol Treatment in the Rat. *Floyd R. Skelton* (introduced by *G. A. Grant*).

11. Clinical, Hormonal and Histologic Study of Human Brucellosis Orchiepididymitis. *F. A. de la Balze, R. E. Mancini* (by invitation), *G. Iacapraro* (by invitation), *F. Arrillaga* (by invitation) and *E. A. Molinelli* (by invitation).

12. The Diagnostic Value of Pneumoperitoneum in Sex Endocrine Disorders. *H. S. Guterman, R. H. Kunstadter* and *A. S. Tulsky* (by invitation).

13. Transvaginal Peritoneoscopy—A New Instrumentality for Evaluation of Ovarian Functions. *A. R. Abarbanel.*

II. 2:00 P.M. ACTH AND ADRENALS (*Red Lacquer Room*)

Chairmen: Irvine McQuarrie and Curt Richter

14. The Inactivation of ACTH in Mammalian Blood. *Gregory Pincus, Oscar Hechter* and *Thomas Hopkins* (by invitation).

15. Blood ACTH in Rat and Man. *Katherine L. Syndnor* and *George Sayers.*

16. Dwarfism with Chronic Hypoglycemia and Convulsions. Observations on ACTH and Thyroid. *Matthew M. Steiner* (introduced by *Lawson Wilkins*).

17. Alterations in 17-Hydroxycorticosteroids of Peripheral Blood Following Acute Whole-Body Irradiation of the Dog. *J. Z. Bowers, D. H. Nelson* (by invitation), *R. Bay* (by invitation) and *L. T. Samuels.*

18. Clinical Studies on Eosinophil Rhythm. *Edmund B. Flink* and *Franz Halberg* (introduced by *Irvine McQuarrie*).

19. Hormonal Influences upon the Distribution and Fate of the Eosinophilic Leukocyte. *Jacques Padawer* (by invitation) and *Albert S. Gordon.*

20. An Experimental Andrenogenital Syndrome in the Frog. *Emil Witschi.*

21. Some Factors Affecting the Success of Pharmacological Blockade of Ovulation in Rats. *John W. Everett.*

22. Studies on Pigmentation in Addison's Disease and in ACTH and Cortisone Administration. *Brian H. McCracken* and *Thomas C. Hall* (introduced by *George W. Thorn*).

23. Hormones and Brain Excitability. *Dixon M. Woodbury.*

24. A Comparison of Effects of Single Injections of ACTH and Epinephine upon Adrenal Cortical Activity. *William McK. Jefferies, Alfred K. Bochner* (by invitation) and *Ralph I. Dorfman.*

25. Effects of Desoxycorticosterone Acetate, Cortisone Acetate and Saline on the Bilaterally Adrenalectomized Rhesus Monkey. *Frederick G. Hofmann* (by

invitation), *Ernest Knobil* (by invitation), *Thomas L. Jones* (by invitation) and *Roy O. Greep.*

26. Effect of Cortisone on the Abnormal Intravascular Distribution of Water in Addison's Disease. *Joseph F. Dingman* (by invitation), *David H. P. Streeten* (by invitation) and *George W. Thorn.*

27. A Quantitative Study of the Prevention of ACTH-Induced Sodium Retention by Potassium Salts. *Grant W. Liddle* (by invitation), *Peter H. Forsham* and *Leslie L. Bennett.*

FRIDAY, JUNE 6, 1952

8:30 A.M. Registration (*Red Lacquer Room*)

III. 9:00 A.M. CALCIUM METABOLISM; SUPPORTING TISSUES (*Red Lacquer Room*)

Chairmen: John E. Howard and Ephraim Shorr

28. Alterations in Calcium Metabolism in Patients with Osteolytic Tumors. *O. H. Pearson, C. D. West* (by invitation), *V. P. Hollander* (by invitation) and *G. C. Escher.*

29. Studies Regarding the Mechanism of Bone Disease in the Detoni-Faconi Syndrome. *L. H. Kyle, W. H. Meroney, III* (by invitation) and *M. E. Freeman* (by invitation).

30. The Use of Intravenous Calcium as a Measure of Activity of the Parathyroid Glands. *John Eager Howard, Theda R. Hopkins* (by invitation) and *Thomas B. Connor* (by invitation).

31. Calcium Loss in Paralytic Poliomyelitis and Its Quantitative Relationship to the Development of Demonstrable Osteoporosis. *G. Donald Whedon* and *Ephraim Shorr.*

32. Strontium as an Adjuvant to Calcium in the Remineralization of the Skeleton in Man. *Anne C. Carter* and *Ephraim Shorr.*

33. Refractoriness to Antitetanic Therapy in a Case of Surgical Hypoparathyroidism. *Raymond W. Blohm, Jr.* (by invitation), *Otto Wurl* (by invitation), *James O. Gillespie* (by invitation) and *Roberto F. Escamilla.*

34. Effect of TSH and ACTH on Amino-Acid Metabolism. *D. M. Bergenstal* and *K. Lugibihl* (introduced by *A. T. Kenyon*).

35. Dietary Modification of the Effects of ACTH and Cortisone. *John W. Partridge* (by invitation), *Lenore Boling* (by invitation), *Florence Olson* (by invitation), *Eleanor Kipp* (by invitation) and *Laurance W. Kinsell.*

36. Hormonal Interaction Regulating Granulation Tissue Development. *Matthew Taubenhaus, Bernard Taylor* (by invitation) and *John V. Morton* (by invitation).

37. The Effects of ACTH and Cortisone on the Development of Experimental Exophthalmos in the Guinea Pig. *A. W. Ludwig* (by invitation), *N. F. Boas* (by invitation) and *L. J. Soffer.*

38. Serum Mucoprotein and Mucoproteins of Connective Tissue of the Skin in Generalized Myxedema. *R. E. Mancini* (by invitation), *J. C. Garberi* (by invitation) and *F. A. de la Balze.*

39. The Adrenal Cortex and Sulfur Metabolism. *Joseph W. Goldzieher, William B. Rawls* (by invitation) and *Max A. Goldzieher.*

40. Nucleic Acid Changes in Tissues of Normal and Diphtheria Intoxicated Guinea Pigs Treated with Cortisone. *Leon T. Atlas* and *Kurt Benirshke* (introduced by *George W. Thorn*).

IV. 2:00 P.M. PRESIDENTIAL ADDRESS (*Red Lacquer Room*)

Presiding—Roy G. Hoskins

President Gregory Pincus: "Some Basic Hormone Problems"

V. 2:30 P.M. STEROIDS; ASSAY, METABOLISM AND PHYSIOLOGY (*Red Lacquer Room*)

Chairmen: Roy Hertz and William T. Salter

41. The Effects of Graded Doses of Cortisone on Circulating Eosinophils in the Intact Mouse. *F. Halberg* and *M. B. Visscher.*

42. Bioassay of Adrenal Steroids Based on Eosinophil Counts in Adrenalectomized Mice. A Statistical Study. *Eugenia Rosemberg* (by invitation), *Jerome Cornfield* (by invitation) and *Evelyn Anderson.*

43. Assay of ACTH Activity Based on Eosinopenic Response of Normal Mice. *Robert S. Speirs* (introduced by *R. K. Meyer*).

44. The Assay of 11-Oxycorticosteroids by Means of Liver Reducing Substances in Adrenalectomized Animals. *N. R. Stephenson* and *J. C. Taylor* (introduced by *L. I. Pugsley*).

45. Erucic Acid as the Factor in Rape Oil Affecting Rat Adrenal Cholesterol. *K. K. Carroll* and *R. L. Noble* (introduced by *J. B. Collip*).

46. Utilization of C^{14}-Acetate for Cholesterol and Steroid Hormone Synthesis in the Human. *Leon Hellman, Robert R. Rosenfeld, David K. Fukushima, T. F. Gallagher* and *Konrad Dobriner.*

47. Biogenesis of Androgens in the Human Testis. *K. Savard* (by invitation), *R. I. Dorfman* and *E. Poutasse* (by invitation).

48. Corticosteroid Metabolism in Isolated Perfused Rat Livers. *O. Hechter, M. M. Solomon* (by invitation), *I. A. Macchi* (by invitation), *E. Caspi* (by invitation) and *M. Feinstein* (by invitation).

49. Levels of 17-Hydroxycorticosteroids Following Intravenous Infusion of Epinephrine into Normal Men. *Don H. Nelson, Avery A. Sandberg, J. G. Palmer* and *E. Myles Glenn* (introduced by *Leo T. Samuels*).

50. The Effect of Cortisone on the Excretion of 17-Ketosteroids in Patients with Adrenal Tumor. *Eleanor H. Venning, C. J. Pattee* and *Frances McCall* (by invitation).

VI. ANNUAL DINNER, Friday, June 6, 1952

Gregory Pincus, President, Presiding

6:30 P.M. Cocktails (*Red Lacquer Room*)

Sponsored by the donors of the Awards of the Society: Ayerst, McKenna and Harrison, Ciba, Schering, and Squibb. Attendance limited to holders of dinner tickets.

7:30 P.M. Dinner (*Red Lacquer Room*)

Presentation of The Squibb Institute for Medical Research Award for 1952.
Presentation of Ciba Award for 1952.
Presentation of Ayerst, McKenna and Harrison Fellowship for 1952.
Presentation of reaward of Schering Fellowship for 1951.

Edward A. Doisy,
Chairman of the Committee on Awards

SATURDAY, JUNE 7, 1952

8:30 A.M. Registration (*Red Lacquer Room*)

VII. 9:00 A.M. PITUITARY HORMONES; THYROID (*Red Lacquer Room*)

Chairmen: J. W. Conn and R. L. Noble

51. The Enhancing Effect of Somatotrophin on the Mammary Growth Induced in Rats with Estrin, Progestin and Mammotrophin. *William R. Lyons, Choh Hao Li* and *Ruth E. Johnson* (by invitation).

52. Interaction of Growth Hormone with Thyroxin and other Hormones as Measured by the Tibia Test. *Irving I. Geschwind* and *Choh Hao Li.*

53. Radioactive Growth Hormone Preparations. *Martin S. Keston* (by invitation), *William L. Money, Rulon W. Rawson* and *Albert S. Keston* (by invitation).

54. Significance of Changes in the Activity of Hepatic and Renal *l*-Glutamic Dehydrogenase Produced by Growth Hormone. *Oliver H. Gaebler* and *James C. Mathies* (by invitation).

55. Influence of Adrenal Hormones on Thyroid Function in the Hypophysectomized Rat. *K. E. Paschkis, D. Epstein* (by invitation), *A. Cantarow* and *G. Friedler* (by invitation).

56. Inhibition of Thyrotrophin, Gonadotrophins and Prolactin by ACTH. *Robert W. Brown* (by invitation), *Dixon M. Woodbury* and *George Sayers.*

57. Graves' Disease: Hyperthyroidism or Hyperpituitarism? *Sidney C. Werner, Howard Hamilton* (by invitation) and *Martha Nemeth* (by invitation).

58. Serum Thyrotrophic Hormone Bioassays in Patients Exhibiting Various Thyroid States. *Benjamin Simkin, Paul Starr* and *Charles Hancock* (by invitation).

59. Life Stress and Thyroid Function in Human Subjects. *B. S. Hetzel, D. S. de la Haba* and *L. E. Hinkle, Jr.* (introduced by *Willard O. Thompson*).

60. Adrenal Cortical Response of Thiouracil-Treated Rats to Cold. *Henry H. Freedman* (by invitation) and *Albert S. Gordon.*

61. Effects of Temperature and Surgery upon the Elimination of Radioiodine in the Rat. *W. V. MacFarlane* (introduced by *C. McC. Brooks*).

62. The Enterohepatic Circulation of Radiothyroxine. *A. Albert.*

63. Effect of Amphenone "B" on the Function of the Thyroid, Adrenals, and Testes. *Robert H. Williams* and *John R. Hogness* (by invitation).

64. Acromegaly Followed by Panhypopituitarism. *W. O. Thompson, P. K. Thompson* (by invitation) and *D. M. Mandernach* (by invitation).

VIII. 2:00 P.M. JOINT SESSION WITH THE AMERICAN DIABETES ASSOCIATION (*Red Lacquer Room*)

Chairmen: Arthur R. Colwell and Gregory Pincus

65. Spontaneous Disappearance of Alloxan Diabetes in the Rat After 12–20 Months of the Disease. *Arnold Lazarow.*

66. Multiple Endocrine Adenomas: Report of Eight Cases Involving the Parathyroids, Pituitary and Pancreas. *Laurentius O. Underdahl, Lewis B. Woolner* (by invitation) and *B. Marden Black* (by invitation).

67. Some Further Studies on the Relationship of Adrenal Cortex Hormones to Experimental Diabetes. *Dwight J. Ingle.*

68. The Evidence for Acceleration of Neoglucogenesis from Fat by ACTH, Cortisone, and Related Compounds. *Laurance W. Kinsell, George D. Michaels, Sheldon Margen, Lenore Boling* and *John W. Partridge.*

Intermission

69. The Banting Memorial Lecture: Insulin. *Charles H. Best.*

70. Studies on Steroid Diabetes in Man. *John J. Bookman* (by invitation), *Stanley R. Drachman* (by invitation), *Louis E. Schaefer* (by invitation) and *David Adlersberg.*

IX. 4:30 P.M. ANNUAL BUSINESS MEETING

Seventy-two papers, which are to be read by title, will be printed in final program.

The American Goiter Association

THE 1952 ANNUAL MEETING

The next Annual Meeting of the American Goiter Association will be held in St. Louis, Missouri, May 1, 2 and 3, 1952, at the Hotel Chase. All those who plan to attend are urged to make hotel reservations immediately.

The banquet will be held Friday night, May 2, on the Starlight Roof of the Chase, with the preceding gathering at 6 P.M. in the Zodiac Room. The business meeting will be on Friday, May 2, at 4 P.M.

Dr. Daniel L. Sexton of St. Louis, is Chairman, and Dr. Willard Barlett, Jr. of St. Louis is Co-Chairman of the Local Committee on Arrangements.

(For the program, *see* the March issue of the Journal, page 366)